Introduction to Nanotoxicology

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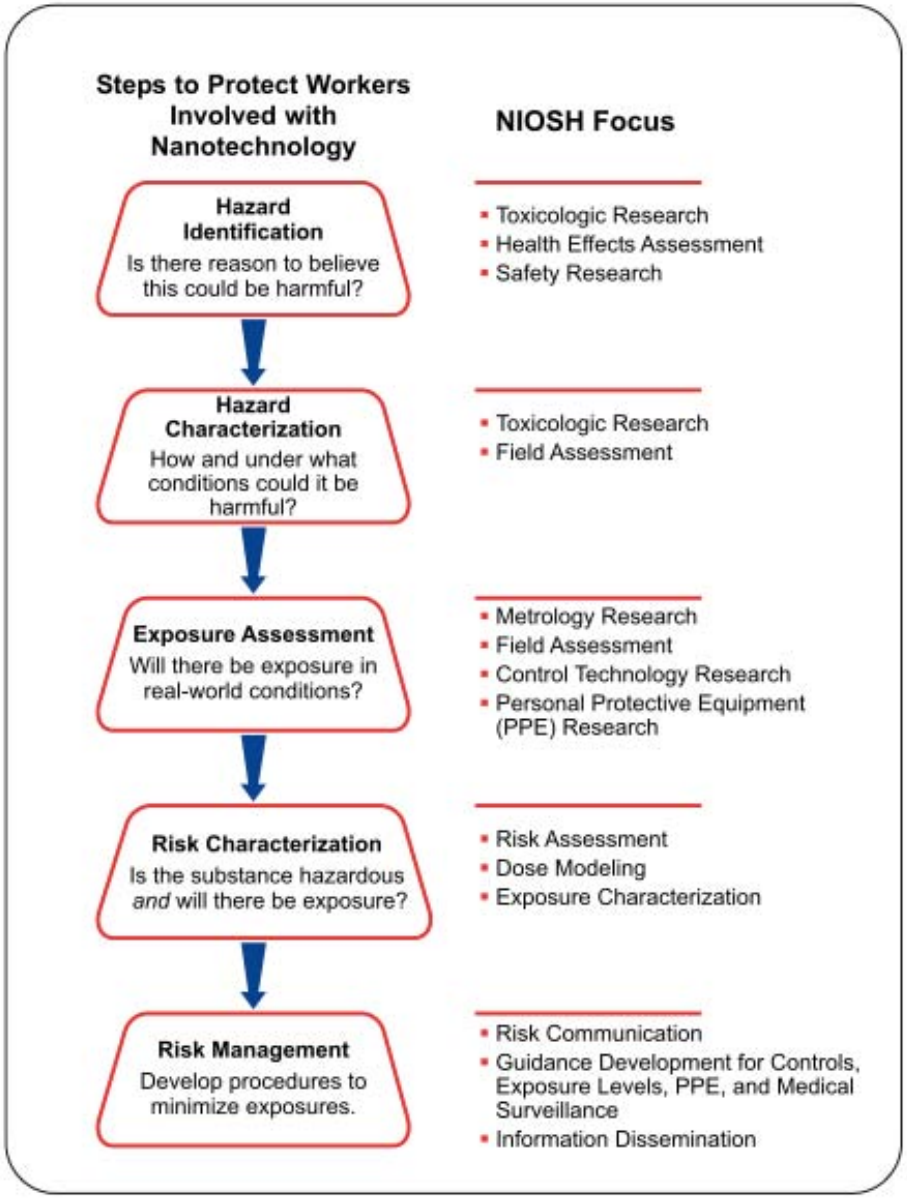
The Foundation for Risk Management



- **Toxicology is the basis for the rest of the process for protecting people from harmful effects of exposure to engineered nanoparticles**
- **The *anticipate* in "anticipate, recognize, evaluate and control"**

Toxicology: Tools and Mechanisms

- In vitro
  - Cell-free preparations
  - Cell cultures
  - Tissue
  - "Tissue surrogates" (complex cell cultures)

- Disadvantages
  - May not represent how cells in an animal would really be exposed
  - Potentially confounded by model used, exposure procedures
  - Doses often very high, physiologically questionable
  - Results may not accurately predict health effects in whole animal

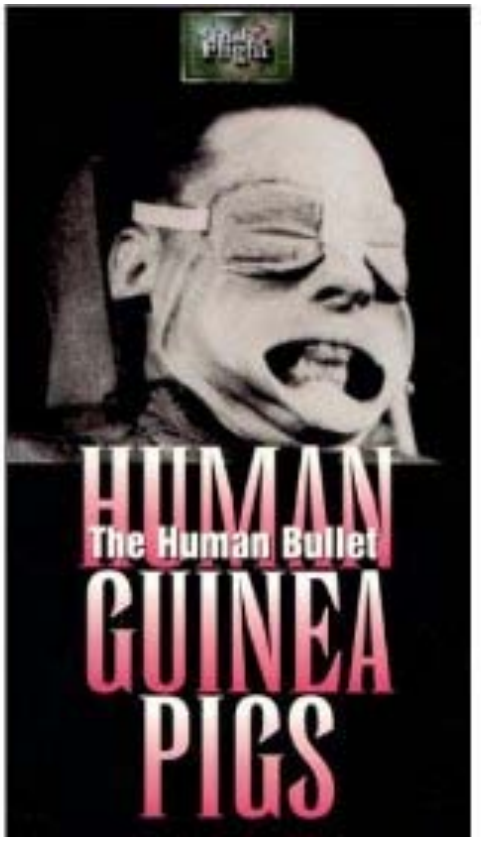
In Vivo Animal Studies

- In vivo animal studies
  - — Acute, sub chronic, chronic
  - — Surrogate exposure procedures
    - Injection, intratracheal instillation, aspiration, implantation
  - — *Real* exposures procedures
    - Ingestion, inhalation, skin contact

- Problems:
  - Rats are not people and may respond differently
  - Animal tests are cruel

Human Studies

- **Human studies**
  - —Experimental exposures
  - —Incidental exposures (accidents)
  - —Epidemiological studies



Why Are We Concerned?

Potential for Novel Toxicity

- **Properties of nanoscale materials may be fundamentally different from bulk materials of same composition**
- **Among the new properties of nanoscale materials may be:**
  - **—*Enhanced* toxicity of toxic materials**
  - **—*New* toxicological properties not seen in bulk material**

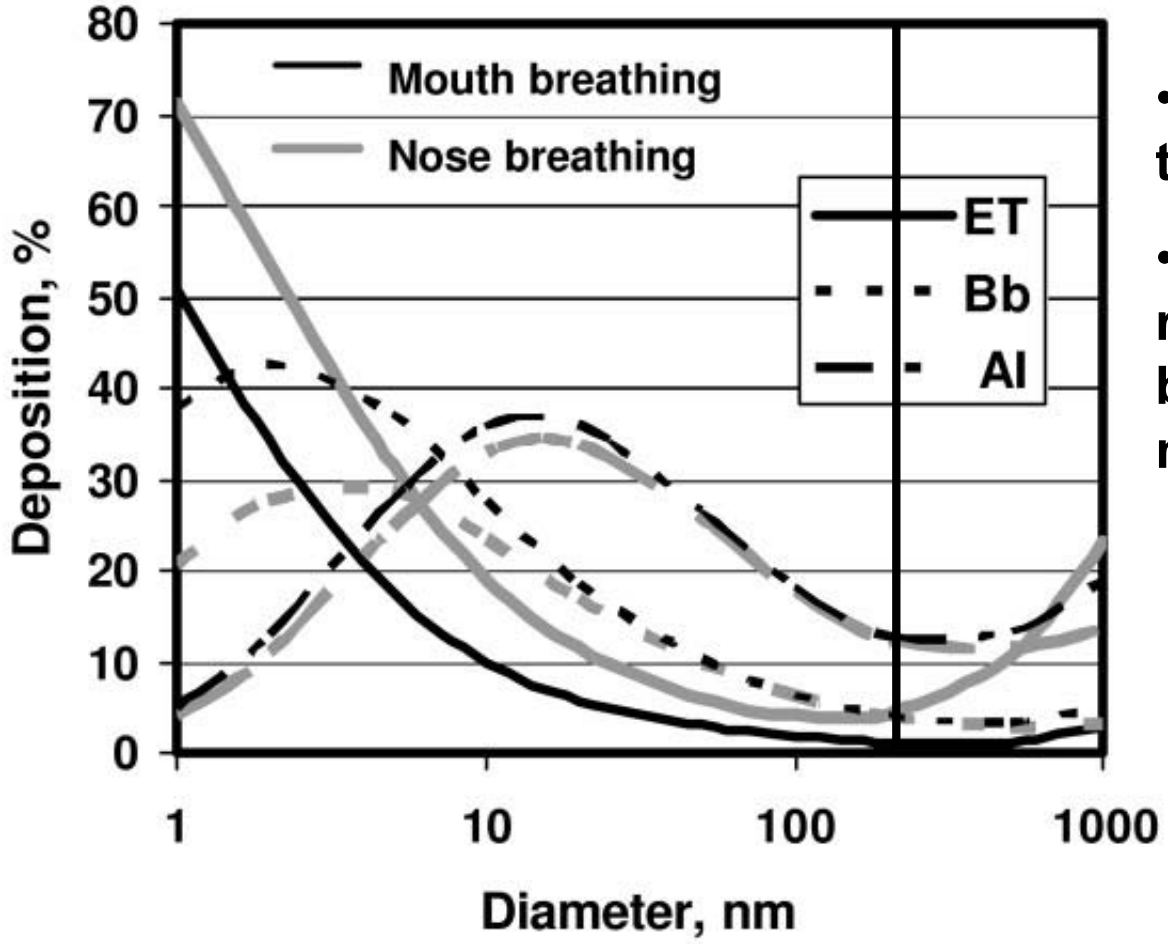
Established Example of Particle Size Dependent Toxicity

- **Free crystalline silica**
  - **Most toxic when <10 μm, for two reasons**
    - **Alveolar deposition of micrometer range particles**
    - **Increased surface area causes oxidative stress via catalysis**
  - **"In conclusion, our data show that quartz elicits DNA damage in rat and human alveolar epithelial cells and indicate that these effects are driven by hydroxyl radical-generating properties of the particles --Schins et al, 2002."**

- **Where in the respiratory tract do nanoparticles deposit?**

http://www4.umdnj.edu/cswaweb/rad_teach/silicosis.html

Respiratory Tract Deposition



- Mostly alveolar down to ~10 nm
- Mostly upper respiratory (nose) and bronchial below ~10 nm!

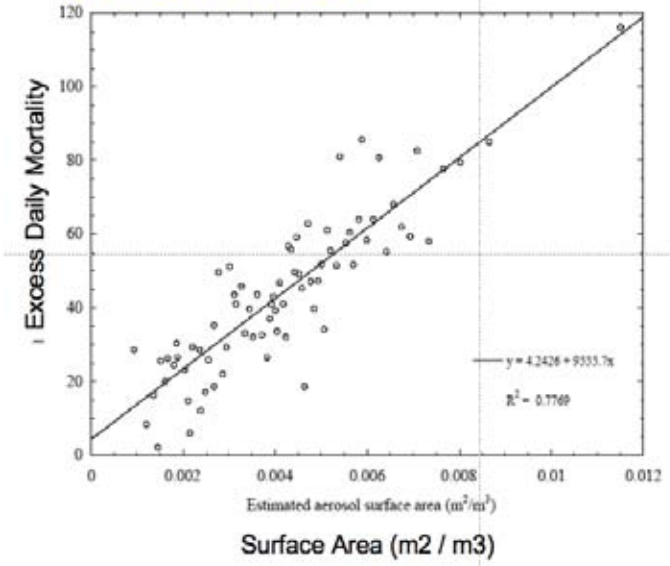
Borm et al. 2006, based on ICRP 66

Ambient Fines and Ultrafines Are Associated With Disease

- **Ambient fines and ultrafines are associated with increased cardiovascular and respiratory events, including death, in susceptible populations**

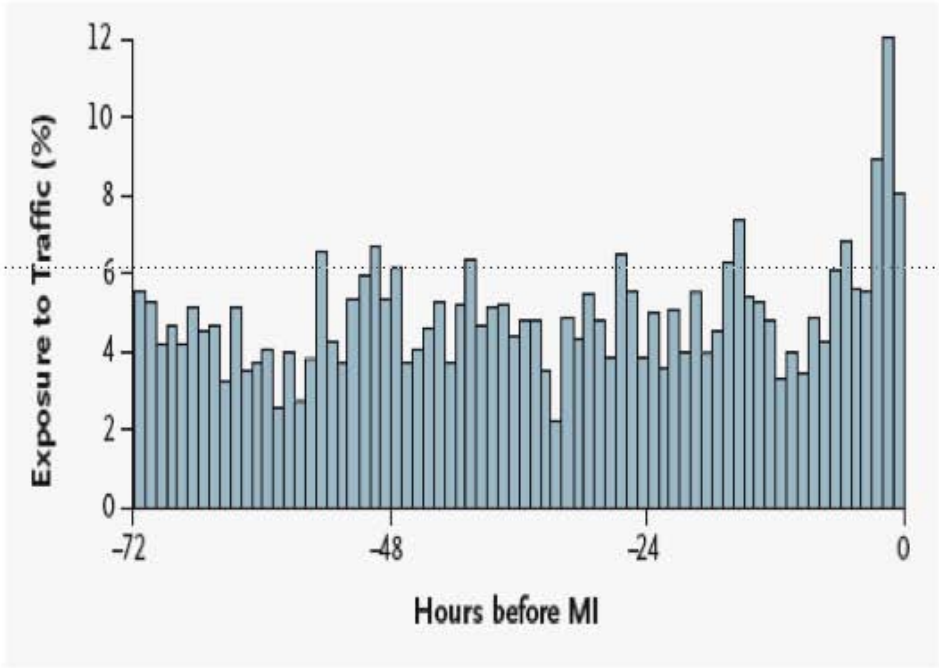
Attack of the Killer London Fog

Mortality Vs. Surface Area Concentration



Ultrafine Exposure Effects Heart

Onset of myocardial infarction (MI) associated with exposure to traffic



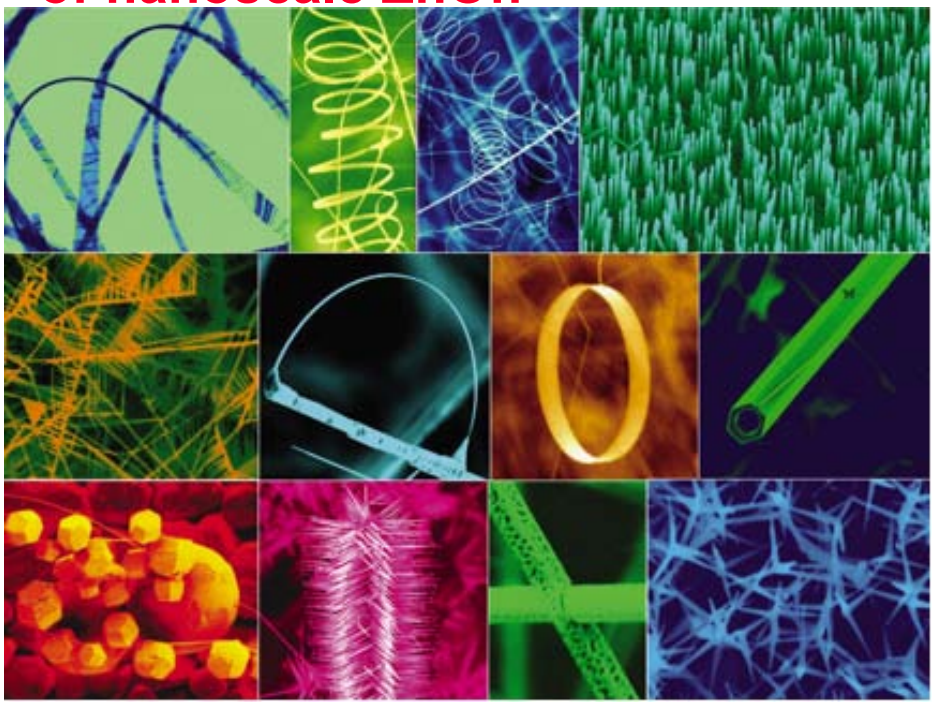
Peters et al., 2004 NEJM

- Increased coagulability of blood Ruckerl et al. (2005)
- Reduced heart-rate variability Liao et al. (1999)
- Increased likelyhood of cardiac arrhythmia
  Peters et al. (2000)

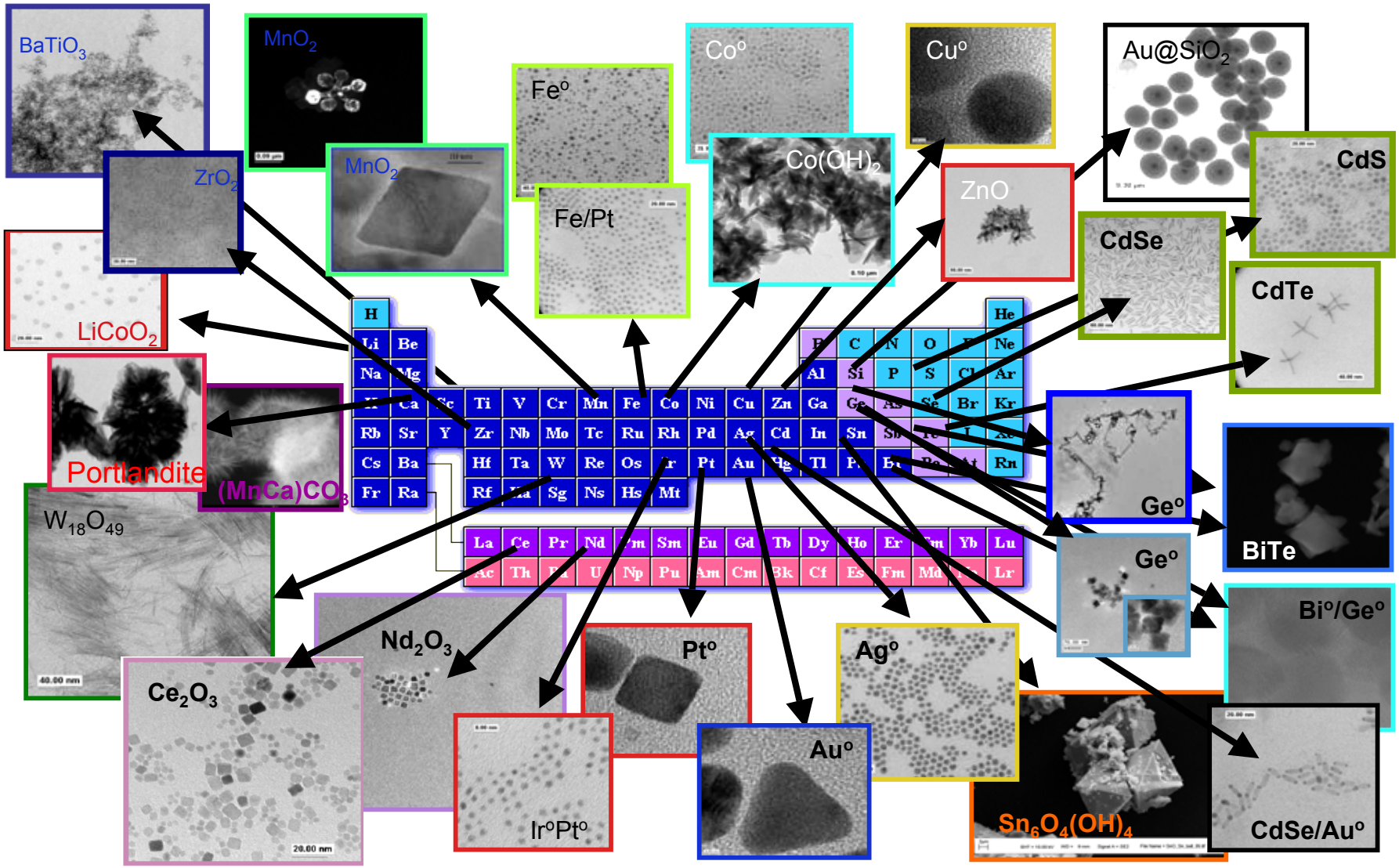
It is not going to be easy to sort out!

- Many variables may effect toxicity
  - Size
  - Shape
  - Chemistry
  - Crystal structure
  - Water solubility
  - Surface area
  - Surface coating
  - Agglomeration state
  - Density
  - Dispersability
  - Porosity
  - Surface charge
  - Conductivity
  - Contaminants
  - Manufacturing method

One chemistry but many forms of nanoscale ZnO!!



Extremely Broad Chemistries



This from one set of labs at the University of New Mexico

Contamination of Materials Leads to False Conclusions

Nanotechnology

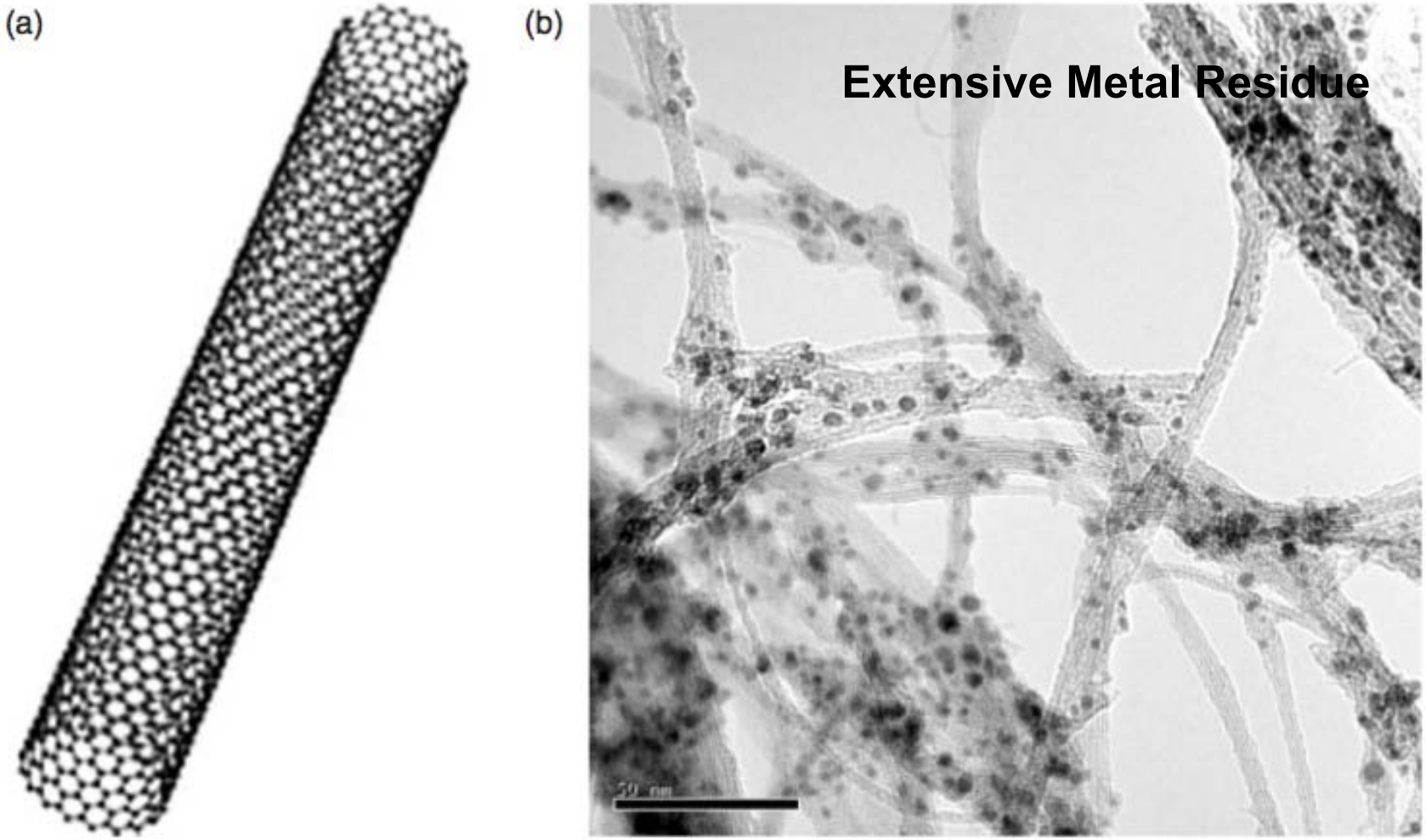
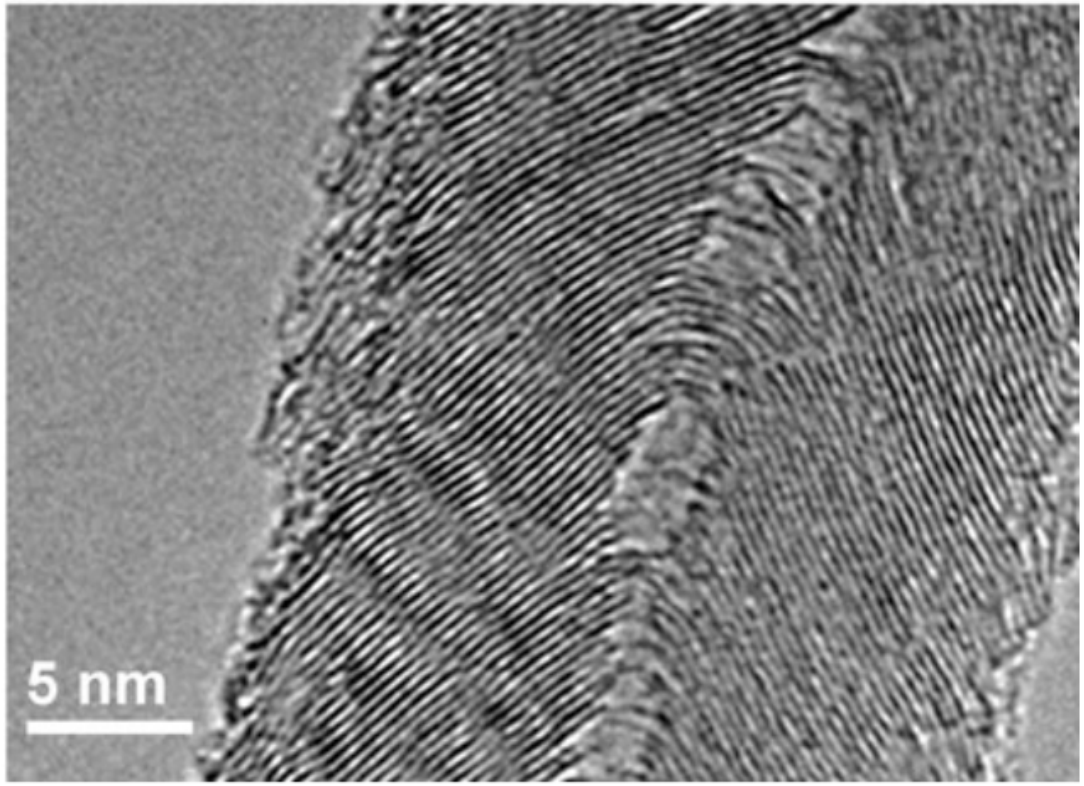


Fig. 1. Single-walled carbon nanotubes. (a) Schematic diagram of a single-walled carbon nanotube © Chris Ewels. (b) Transmission electron micrograph of as-produced single-walled carbon nanotube, showing aligned clusters of nanotubes (nanoropes) and nanometre-diameter metal catalyst particles, used in the production process.

- **From Maynard, 2007**

Nanotube, NOT!!



- Representative "carbon nanotube" from Mitchell et al (2007) inhalation study is in fact a nanofiber.
- Cheap Tubes!
- The authors didn't know the difference!

Problems With Surrogate Dosing Models

- **Artificial dosing methods may cause epiphenomenal effects**
  - **Right–Animals choked to death by CNTs instilled in their lungs**

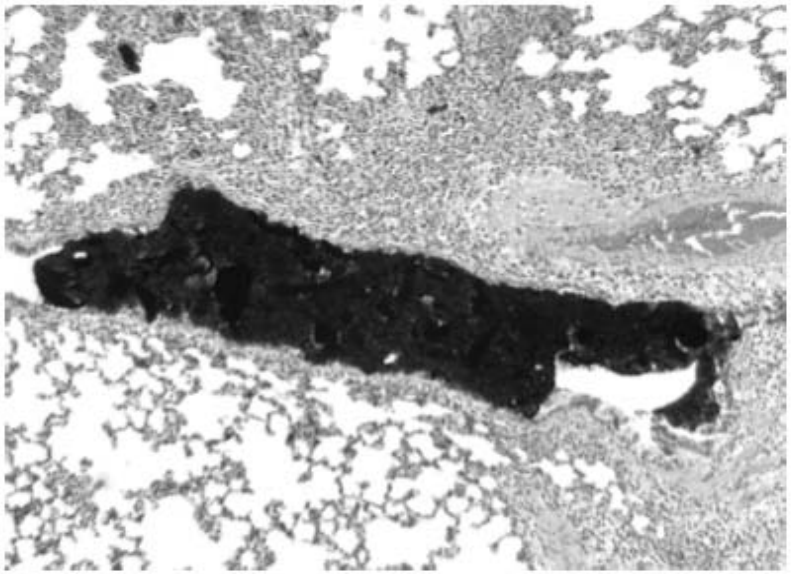


Figure 1. Light micrograph of lung tissue from a rat exposed to 5 mg/kg SWCNT (a few hours after exposure). The major airways are mechanically blocked by the SWCNT instillate. This lead to suffocation in 15% of the CNT-exposed rats and was not evidence of pulmonary toxicity of SWCNT.

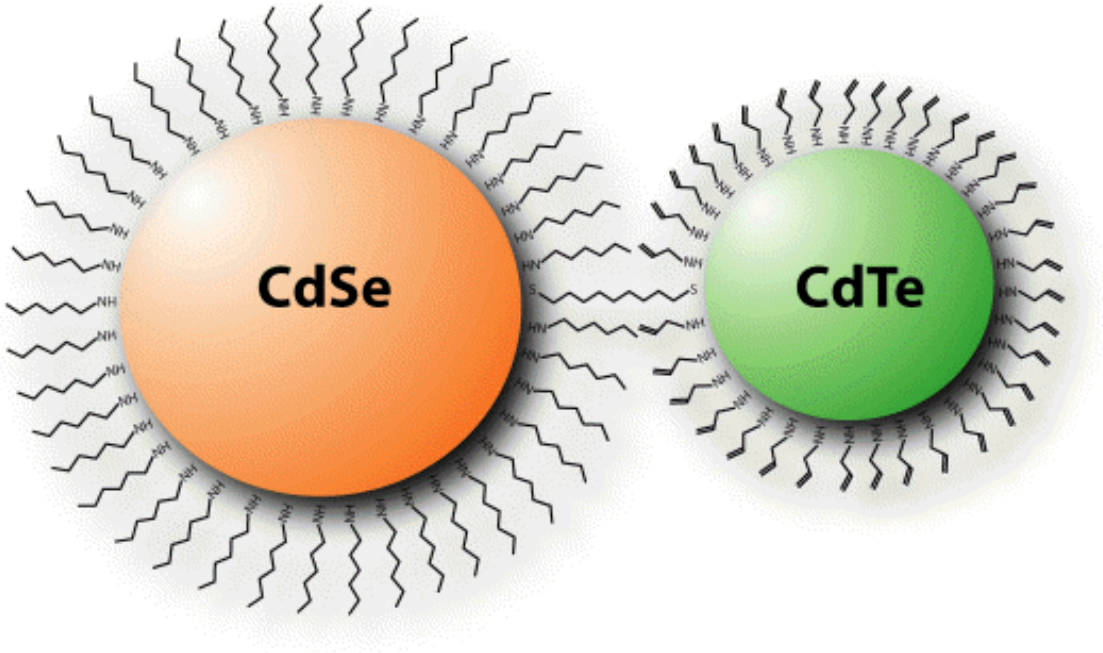
Mechanisms of Toxicity

Drivers of Toxicity

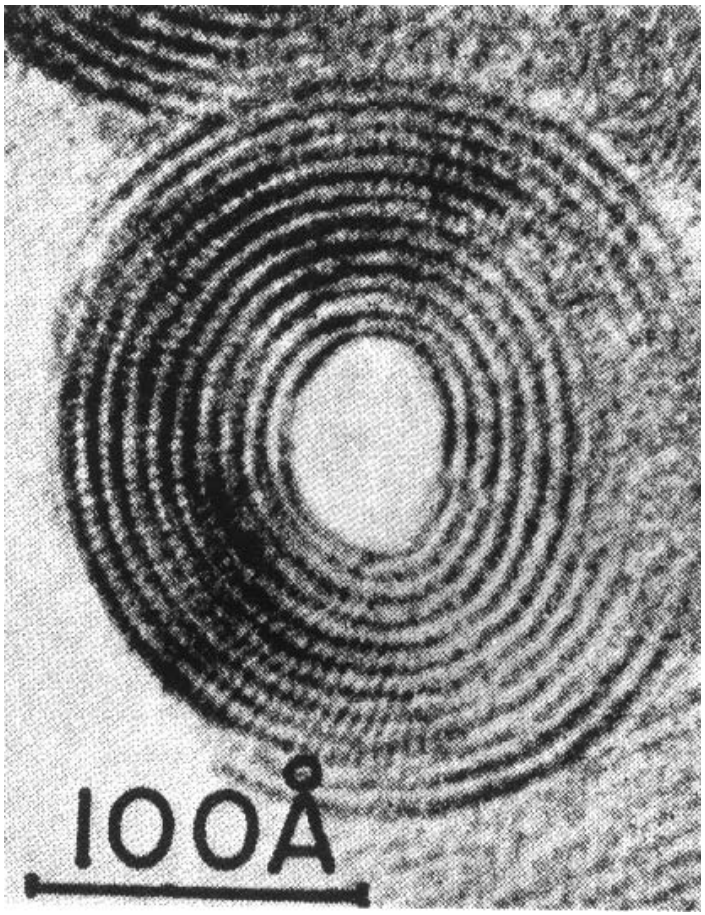
- Intrinsic elemental (chemical) toxicity
- Morphology-driven toxicity
- Surface reactivity driven toxicity

Chemistry Driven Toxicity

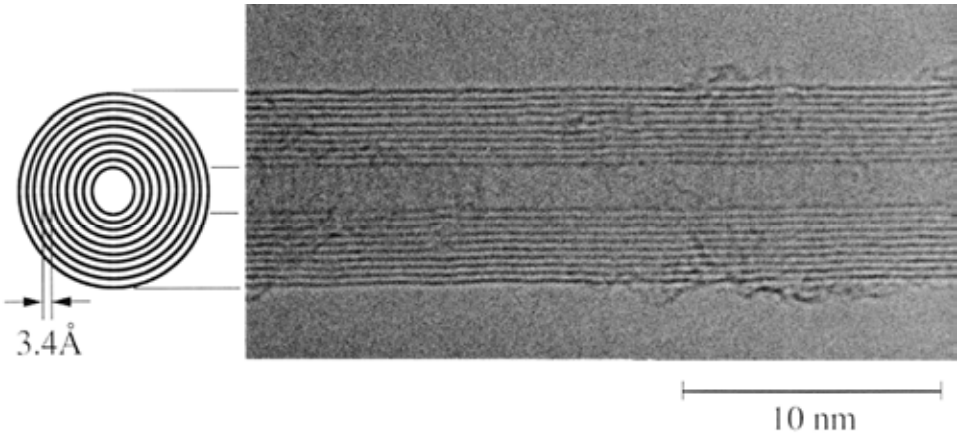
- **Semiconductor nanocrystals, quantum dots**
  - **—Se, Cd, Pb, Te, Zn, S, Ga, Sb, As, In**
  - **—Elemental toxicity (ions) *partially* explains quantum dot toxicity**



Morphology Driven Toxicity



www.gly.uga.edu/schroeder/geol6550/CM07.html



Two similar appearing *nanotubes*

- **Chrysotile asbestos (left)**
- **Multiwall carbon nanotube (above)**

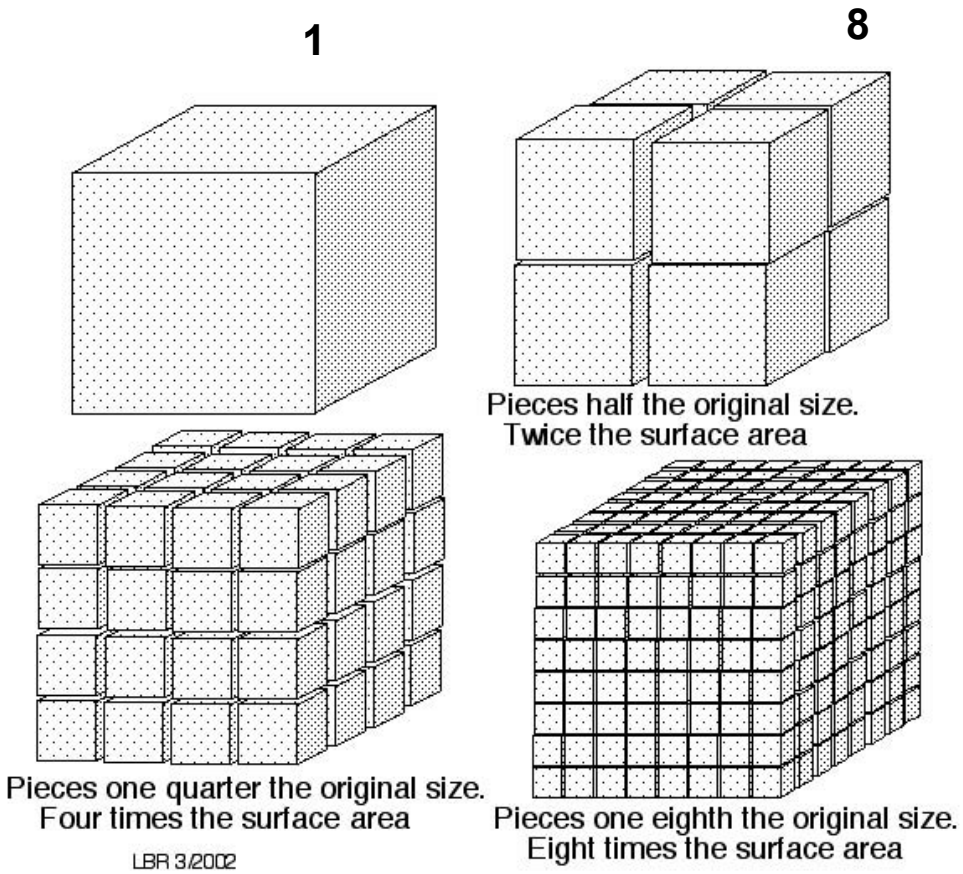
Similar toxicity?

Morphology-Driven Toxicology
Fiber Toxicology

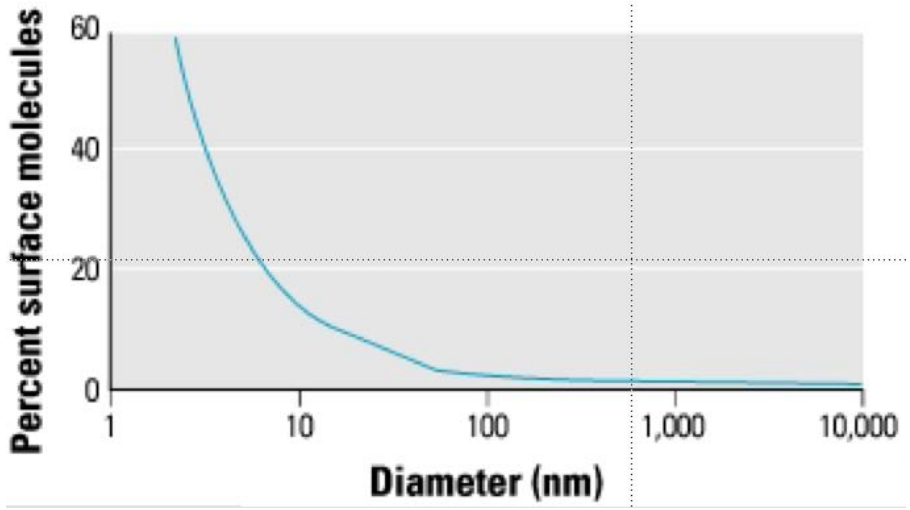
- **Key factors contributing to toxicity:**
  - **Diameter < 1000 nm**
  - **Length >5,000 nm:**
  - **High biopersistance**
  - **Poor pulmonary clearance**

- **More on carbon nanotubes to come**

Surface Area Driven Toxicity



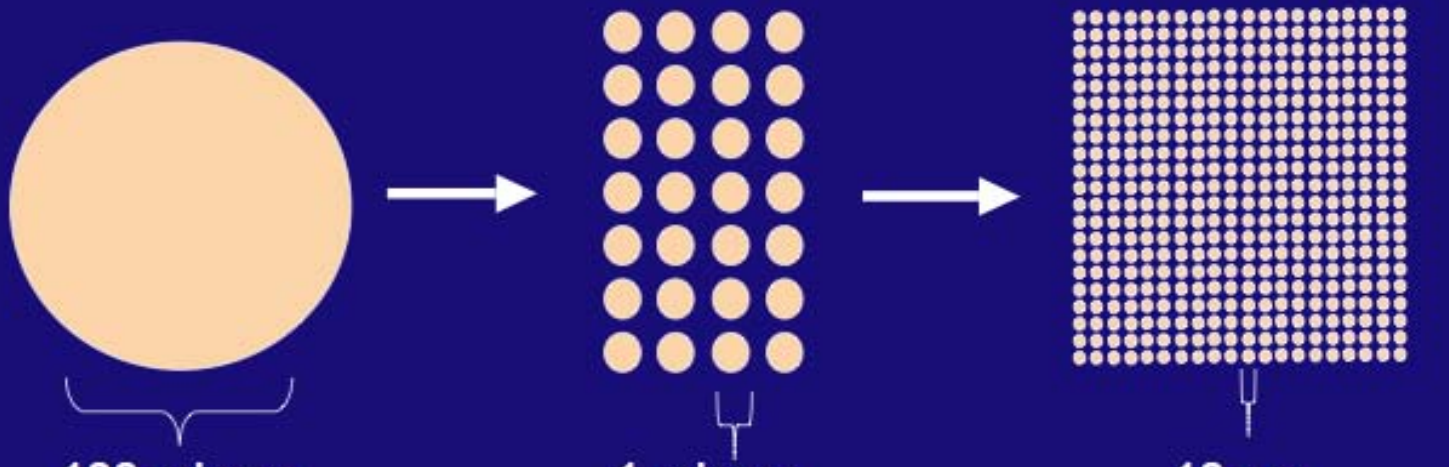
- Nanoparticle surface area is huge
- More surface area = more catalysis = more reactive oxygen species = more cell injury



•www.gly.uga.edu/railsback/1121WeatheringArea.jpeg

What's the right dose measure?

One milligrams of quartz sand:



One hundred particles

One billion particles

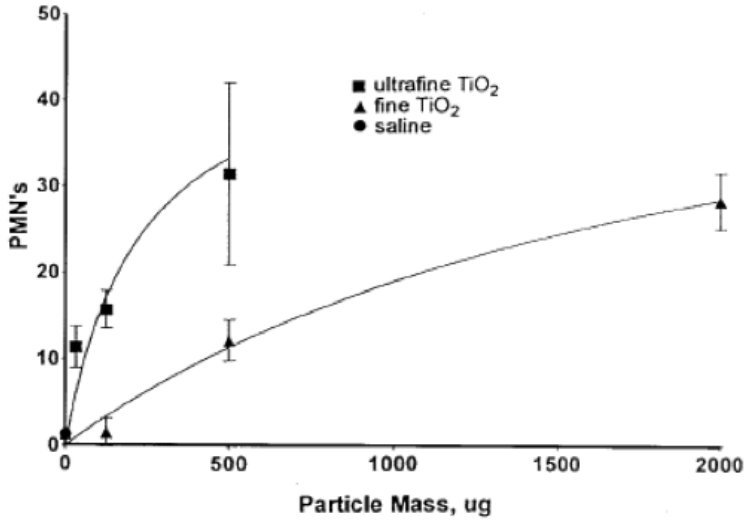
One hundred trillion particles

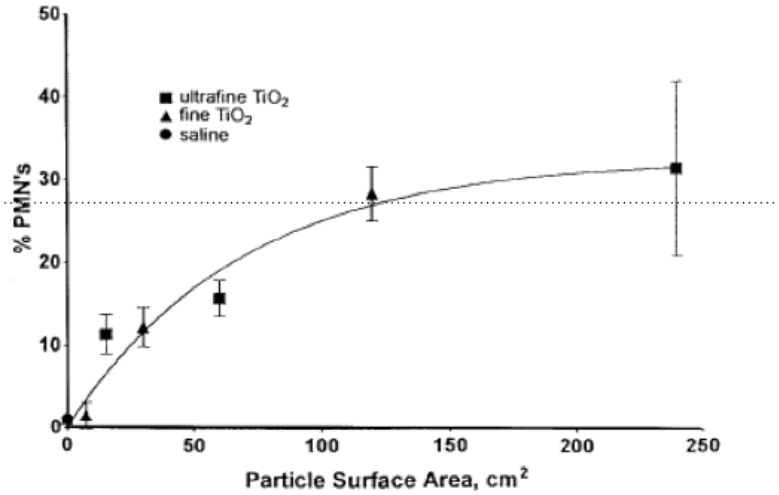
Alison Elder, U Rochester

Surface Area May Be Critical Metric

Especially for materials that are of low solubility and low elemental toxicity, e.g. Ti, Zr, Ba, Au, polymers, fullerenes

- **Toxicity of ultrafine TiO2 appears much higher than fine TiO2 *per unit mass***
- **Toxicity is equivalent when *surface area* is the exposure metric**

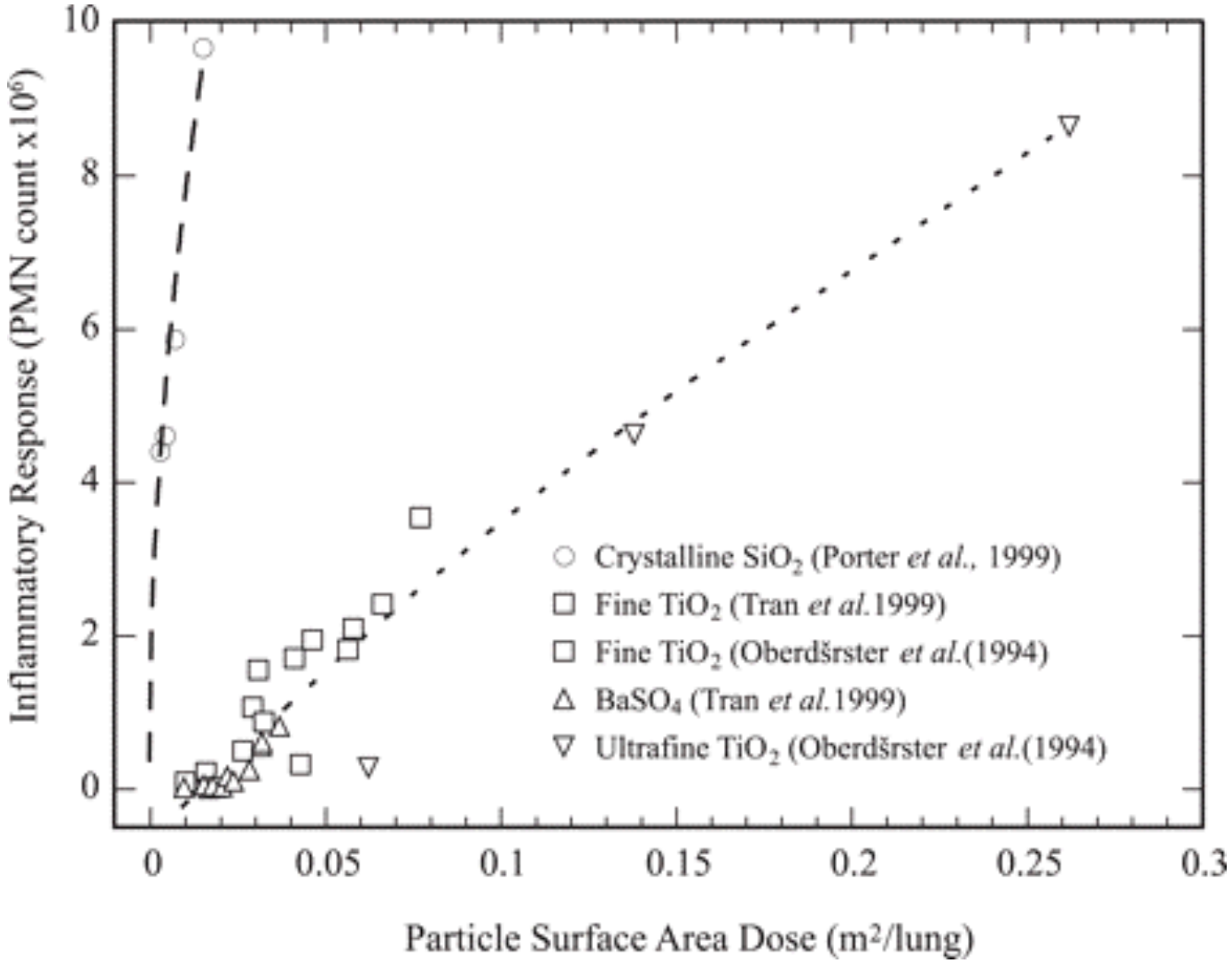




Measured polymorphonuclear neutrophils in lung lavage fluid, an index of inflammation

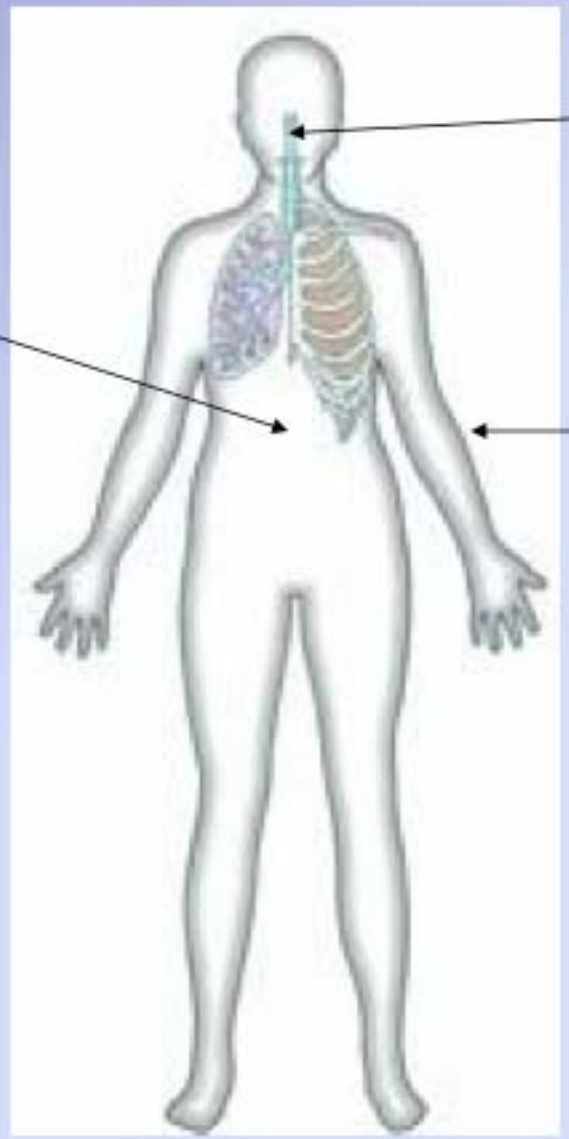
Oberdorster, Int Arch Occup Environ Health. 2001 Jan;74(1):1-8.

Dose of Perspective



- **Nanoquartz is much more toxic than other low solubility nano-oxides**

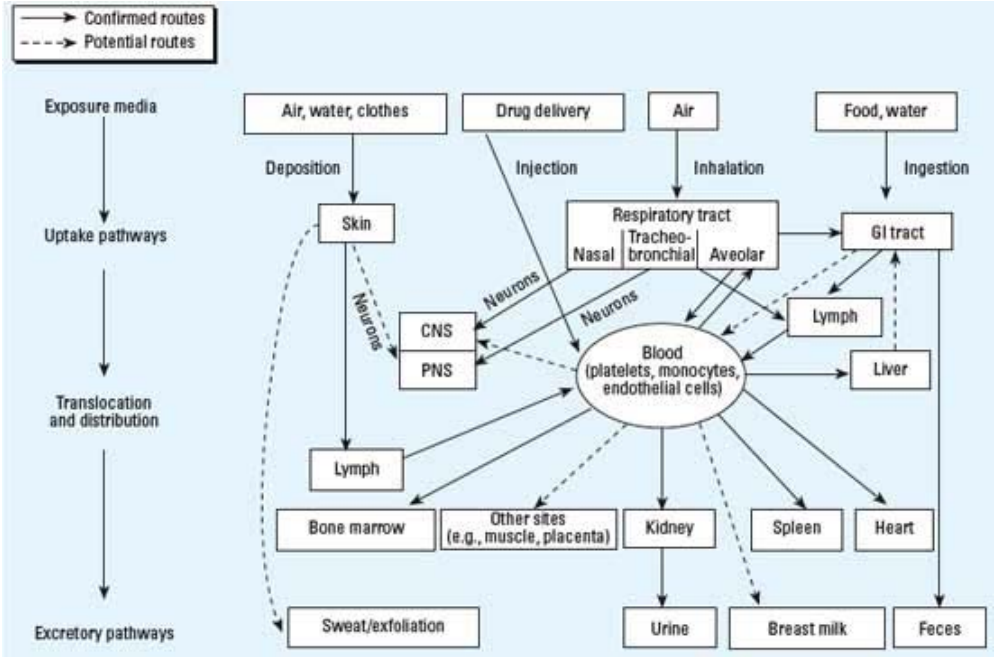
Exposures to Nanomaterials: Most Likely Routes



From: Borm et al., 2006

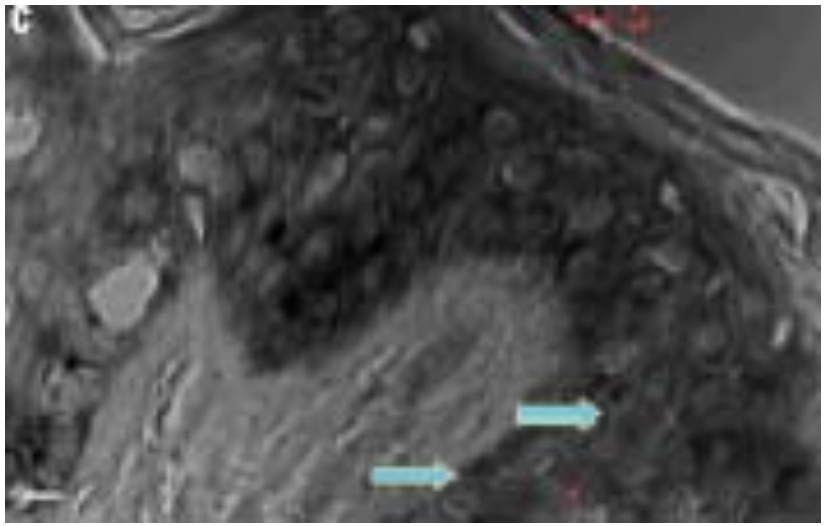
Distribution Across Anatomical Barriers

- **Nanoparticles may bypass normal body barriers to distribution!**
  - **— Through intact skin–maybe**
  - **— Through the GI epithelium–yes!**
  - **— Through the respiratory tract epithelium–Yes**
  - **— Up along nerve axons from the nose to brain–Yes**
  - **— Across the placenta! Barrier–Possibly**
  - **— Through the blood-brain barrier–Maybe**
  - **— Through the blood-testes barrier–Probably**

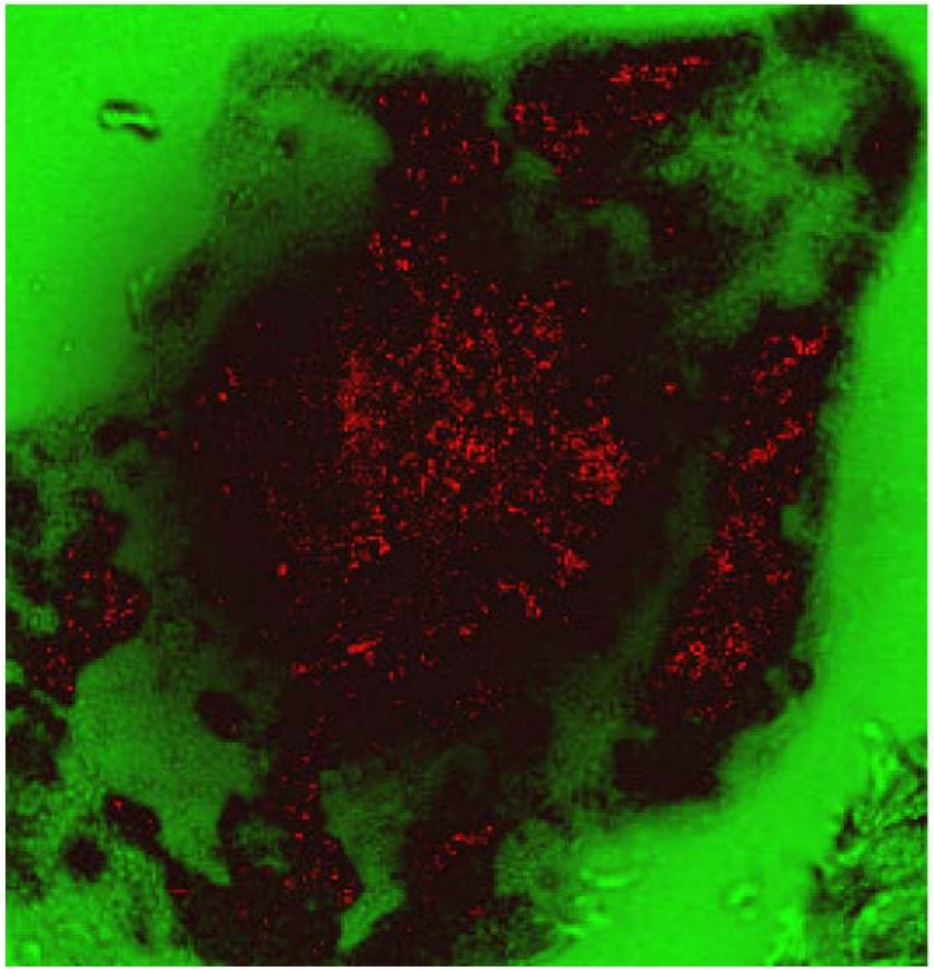


Can Nanoparticles Penetrate the Skin?

- Static application models never show penetration
- Flexed skin models show some penetration
  - —After flexion/ extension of skin, smaller fluorescent dextran beads 500, 1000, 4000 nm penetrate epidermis reaching dermis
    - (Tinkle, et al. Environ Health Perspect. 2003; 111:1202-1208)



TiO2 certainly gets into hair follicles

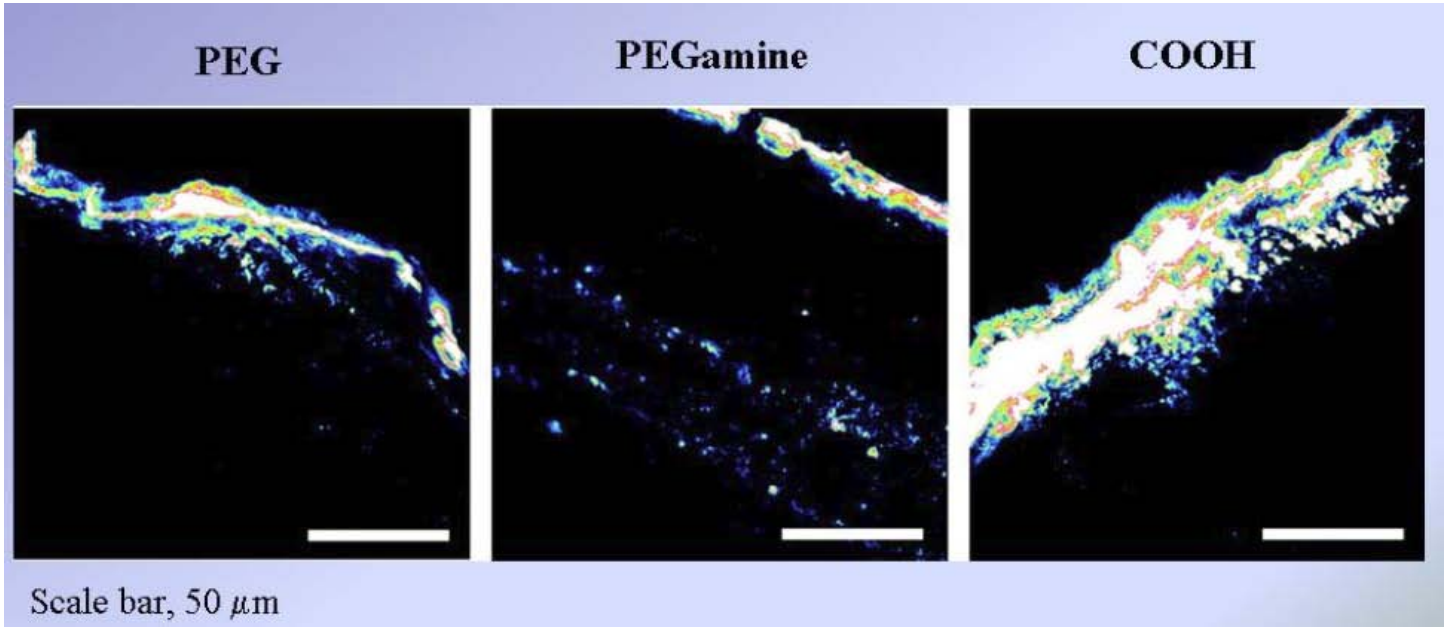


- **Red fluorescence indicates TiO2 nanoparticles (sunscreen) trapped in hair and oil pores in skin.**
- **Used "tape stripping" on live humans**

- **Stratum corneum**: presence of titanium dioxide NP
- **Follicles:** in approximately 10% of follicles fluorescence was observed
- **Interfollicular epidermal tissue below the stratum corneum**: absence of titanium dioxide NP

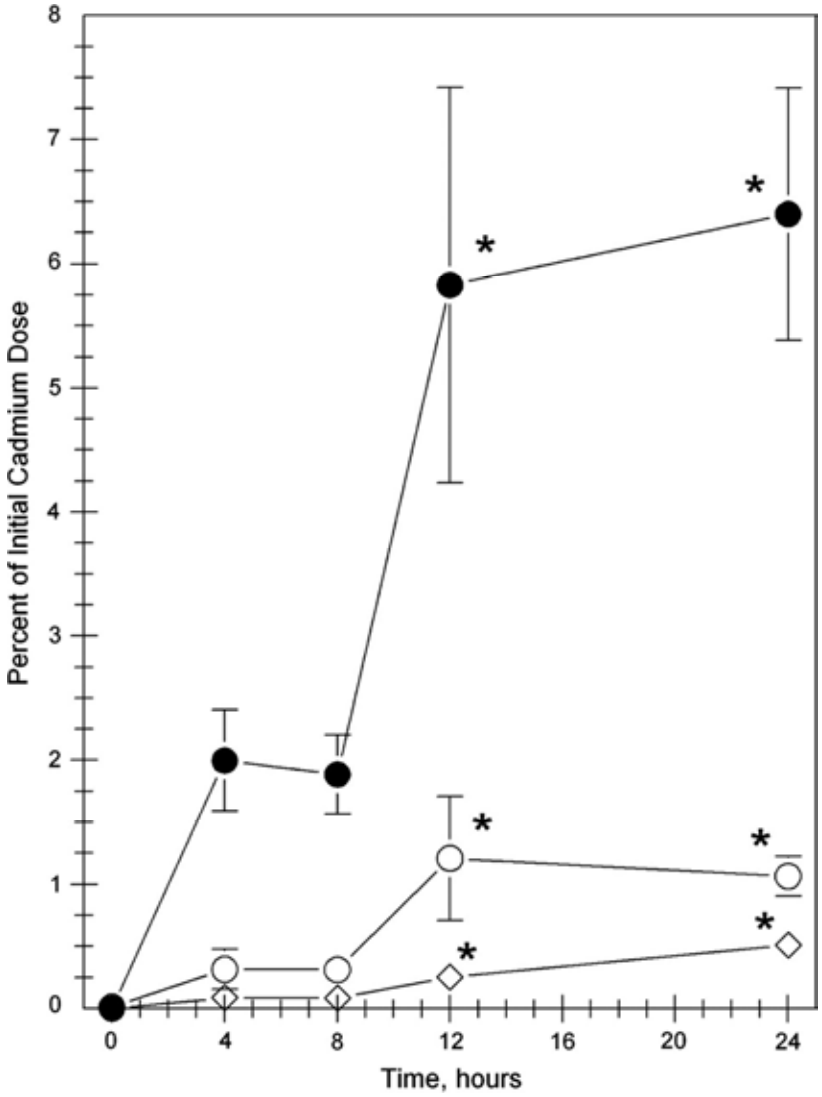
Quantum Dots

- **CdSe quantum dots can penetrate damaged skin to a limited extent (Ryman-Rasmussen 2006, Monteiro-Riviere 2008)**



- **Penetration is limited, and varies strongly based on the surface charge of the QDs**

QDs injected into the skin do circulate

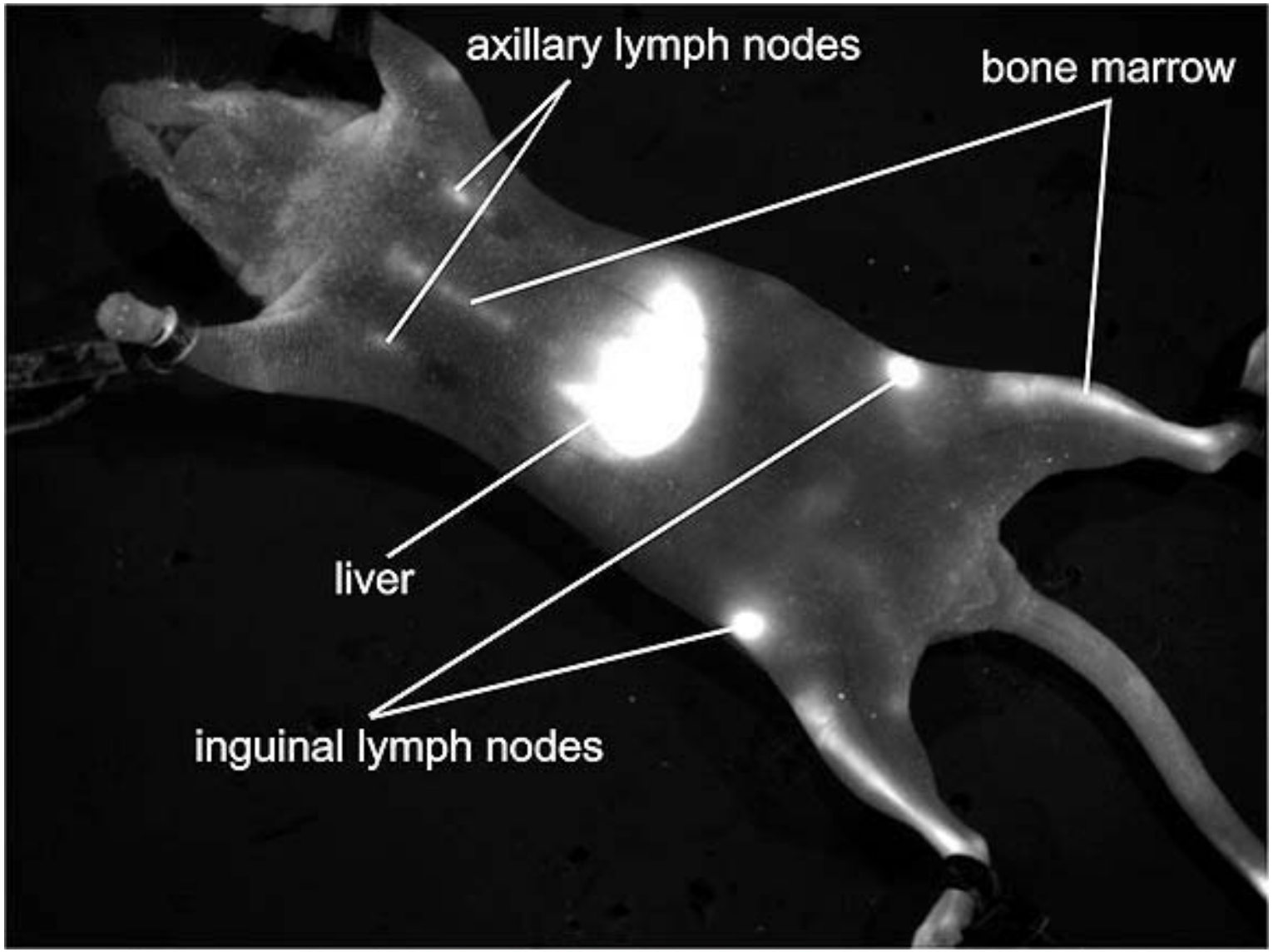


After 24 hours

- **Liver**
- **Lymph**
- **Kidney**

•Migration of Intradermally Injected Quantum Dots to Sentinel Organs in Mice, Gopee et al, 2007

Bioaccumulation– Quantum Dot Mouse



•B. Ballou, BC Lagerholm, LA Ernst, M P.Bruchez,AS Waggoner; Bioconjugate Chem. 2004, 15, 79-86

Systemic Effects and the Question of Translocation of Nanoparticles from the Lung

To blood, lymph and beyond!

Why do we care about translocation?

- **Bad Karma!**
  - —Translocation of asbestos fibers into the mesothelium leads to mesothelioma
  - —Translocation of CDNP may be basis for some of the systemic toxicity these materials induce, including cardiovascular toxicity

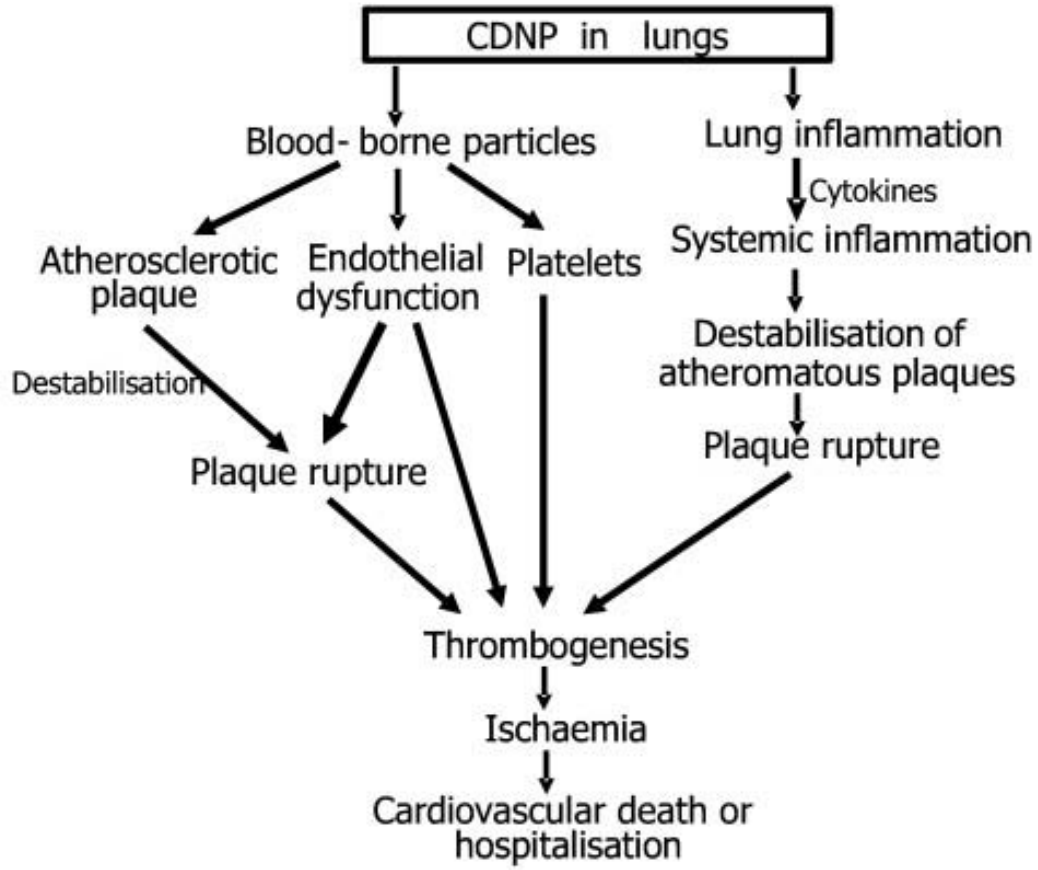
Can Inhaled Fibers Migrate To the Pleura to Potentially Cause Mesothelioma?

Persistent Pulmonary Fibrosis, Migration to the Pleura, and Other Preliminary New Findings after Subchronic Exposure to Multi-Walled Carbon Nanotubes

Multi-walled carbon nanotubes, known as MWCNTs for short, are a type of engineered nanomaterial that shows promise for various applications. These include the potential for creating stronger, more durable building materials; improving cancer therapies; creating more efficient means of energy generation, storage, and transmission; and speeding computer processes. However, as with other types of engineered nanomaterials, the potential occupational health implications of MWNCTs are not well understood at this emergent stage of the technology. A broad group of health and safety practitioners and business observers have agreed that research is vital for determining if MWCNTs pose a health risk for workers engaged in their production and industrial use, and for informing the responsible development of this technology. There is general agreement that this issue must be approached in a proactive manner with good research in order for society to benefit from the many promises this new class of materials has to offer.

NIOSH, 2009

Translocation may not be Required for Some Systemic Toxicity



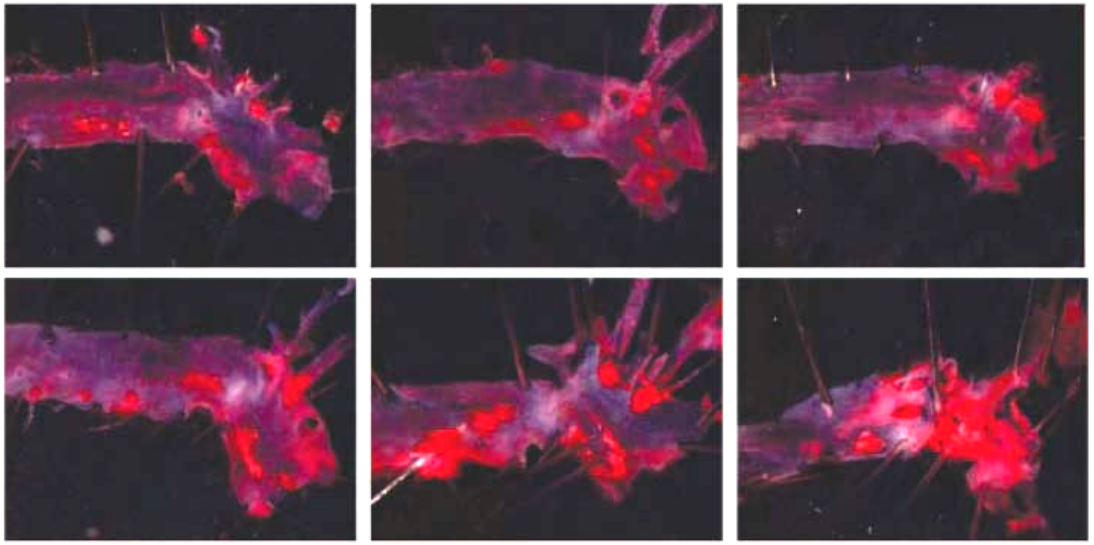
May or may not require that particles translocate from lung

Donaldson et al 2005

Cardiovascular Toxicity of CNTs Instilled In The Respiratory Tract

- Li Zheng of NIOSH, March 2007
  - Intrapharyngeal dosing of SWCNT in mice resulted in oxidative stress in aorta and heart tissue and damaged mtDNA in aorta
  - Accelerated atherosclerosis

Control aortas



SWCNT exposed aortas

Lung Translocation

- **Kreyling studied the transport of radioactive irridium particles through lung**
- **80 nm--0.1% translocated through the lung and ended up in the liver**
- **15 nm--0.5% translocated, a 5x increase**

Comclusions:

•“ Only a small fraction of intratracheally instilled UFPs can pass rapidly into systemic circulation”

•Smaller nanoparticles translocate faster than larger particles

• Pulmonary inflammation seems to play a major role in enhancing the extrapulmonary translocation of particles.

MnO Translocation from Nose to Brain

- Inhalation of nano MnO resulted in increase in brain Mn levels, particularly in the olfactory bulb
- Might be due to brain uptake from the blood of ingested or dissolved Mn, but hard to explain the specific targeting of the olfactory bulb
- Suggests direct olfactory transport to olfactory bulb

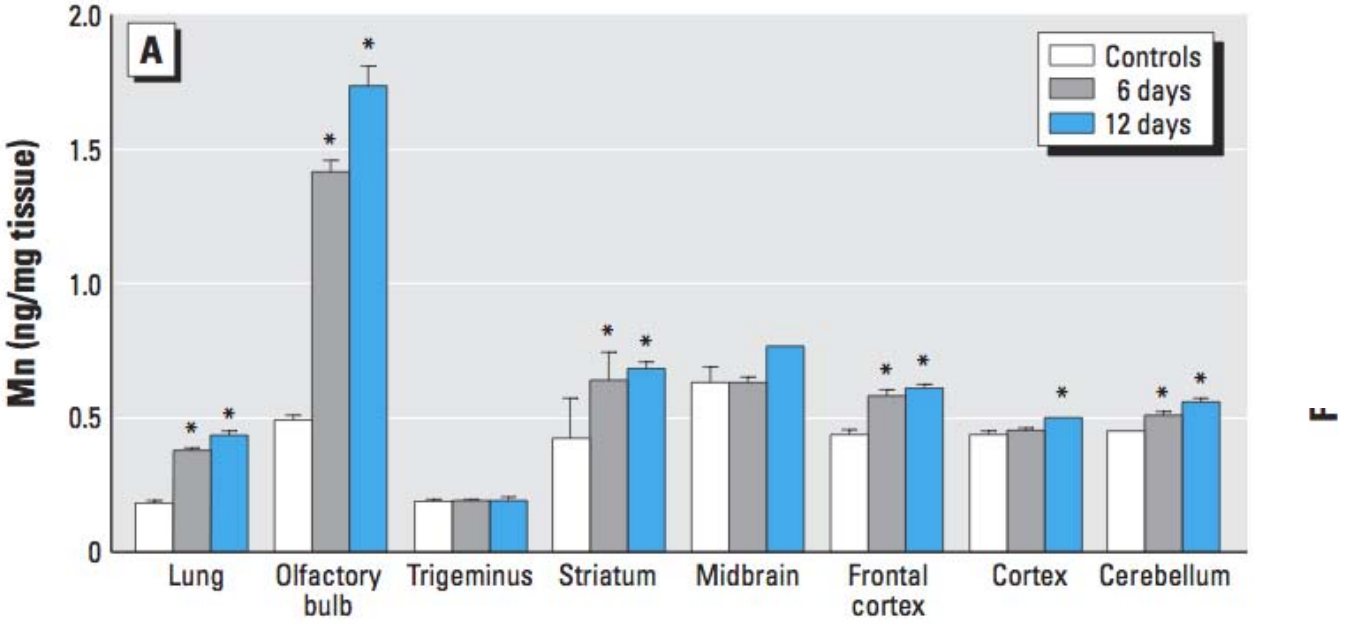
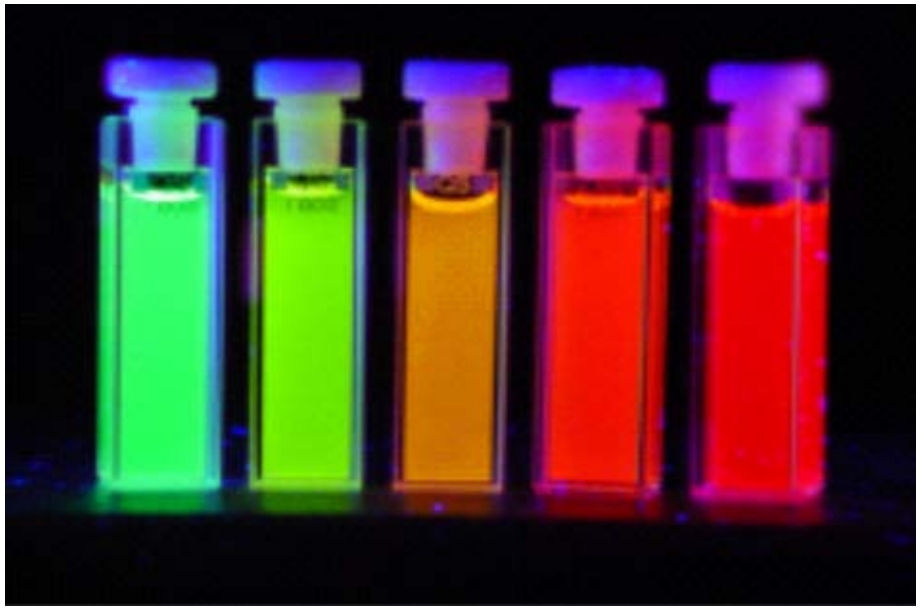
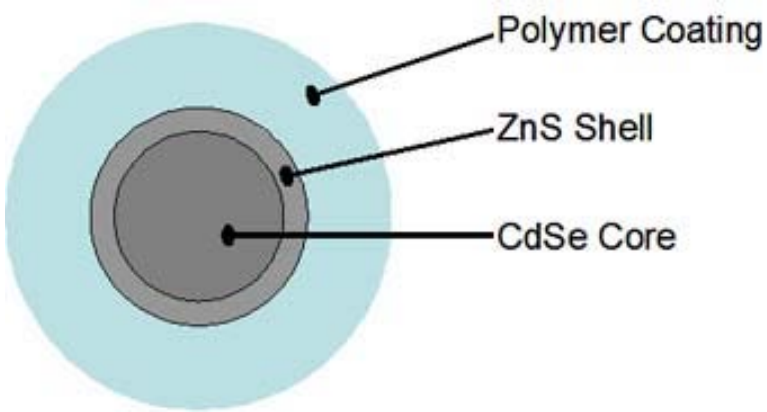


Figure 1. Mn and Fe contents in lung and brain tissues after 6 and 12 days of inhalation

Quantum Dots

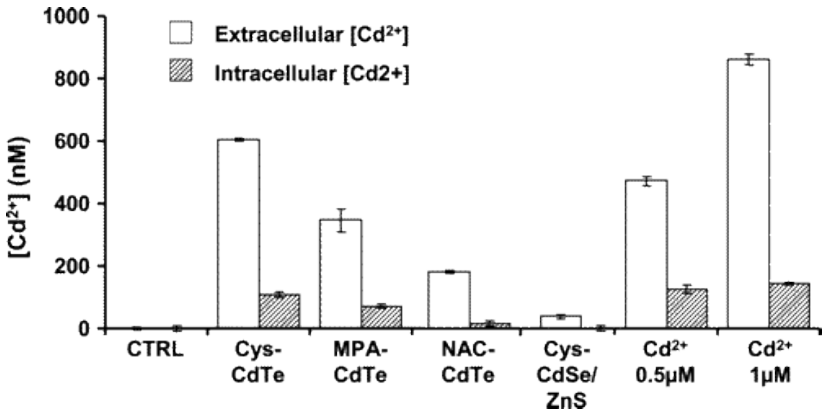




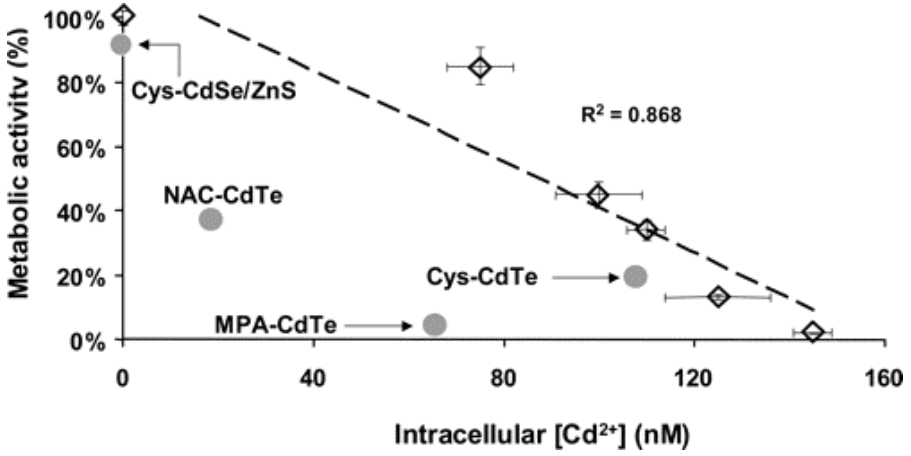
- Semiconductors a few nm in size
- CdSe, CdTe, GaAs, others
- Fluorescence is a function of size
- Potentially very useful in medicine and science
- All start with metal core, some have metal shells then usually plastic coating, sometimes ligands attached to core

Quantum dots Cho et al 2007

- Several studies suggested that the cytotoxic effects of quantum dots (QDs) may be mediated by cadmium ions (Cd2+) released from the QDs cores.
- But there is another mechanism in play too…

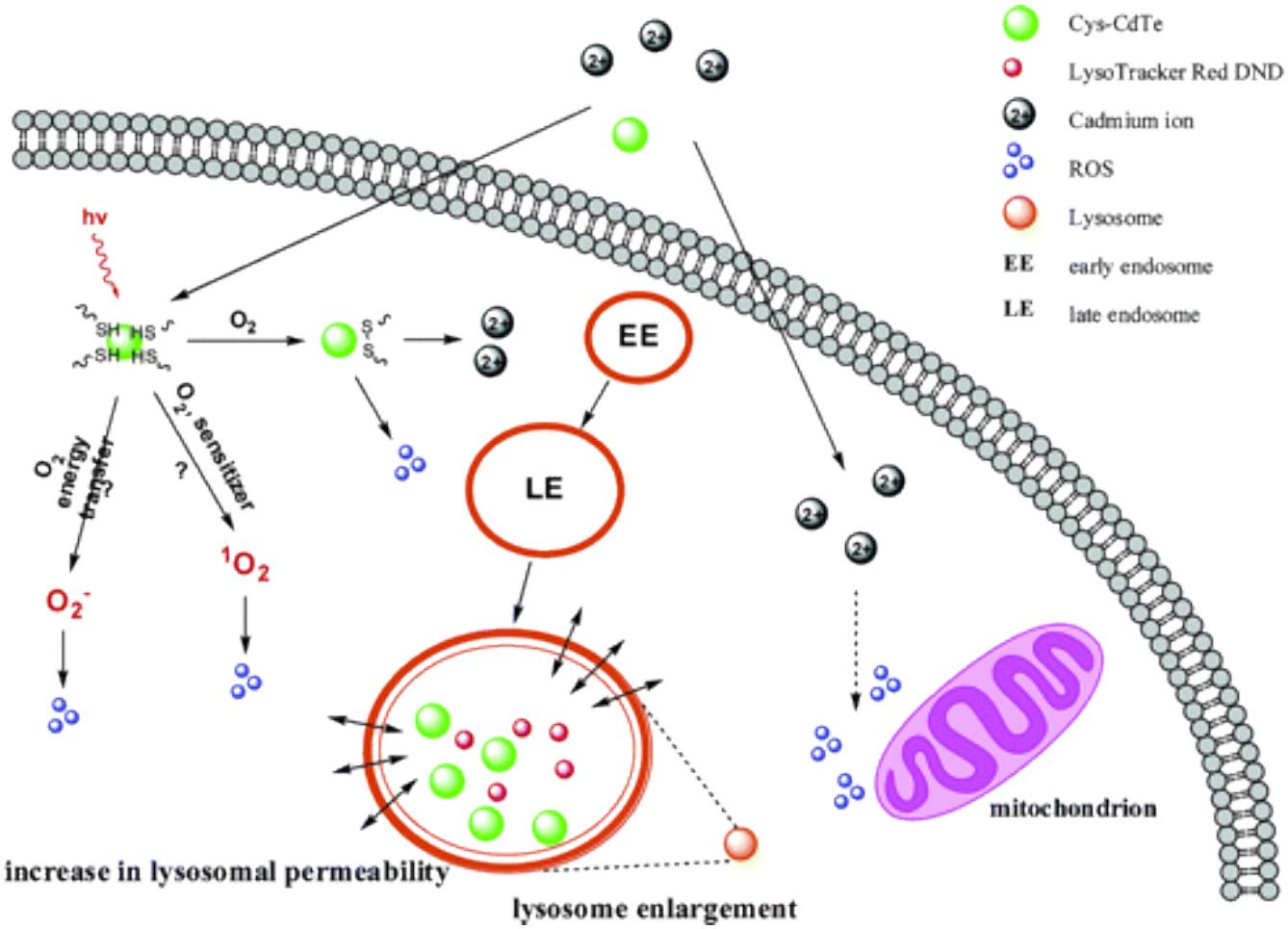


•Different coatings on QDs result in different Cd2+ ion release



•Toxicity does not vary with Cd ion levels!

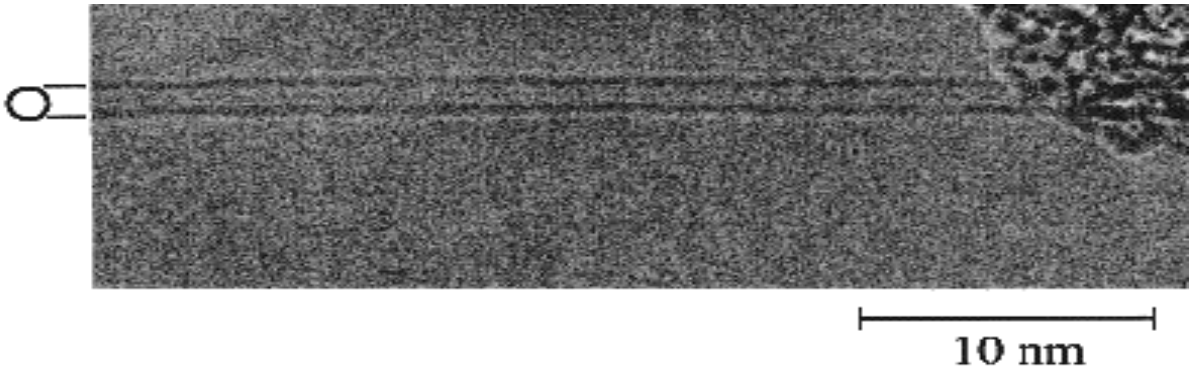
Duel Mechanisms: CdTe Toxicity



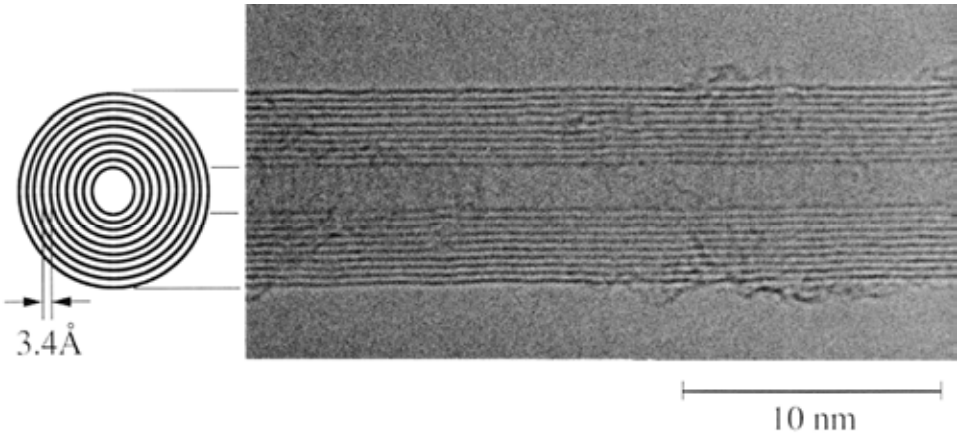
- **ROS derived from Cd ion and intact particles**

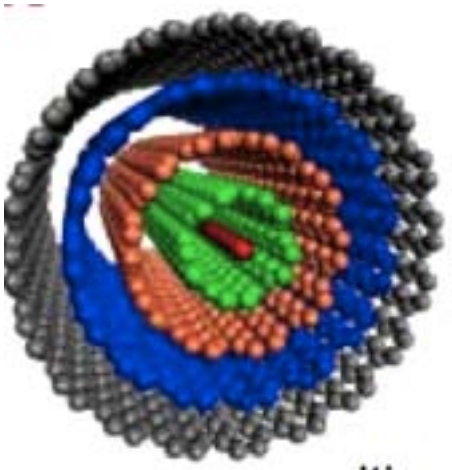
Single Tube Electron Micrographs

Single-Walled Carbon Nanotubes (SWCNTs)

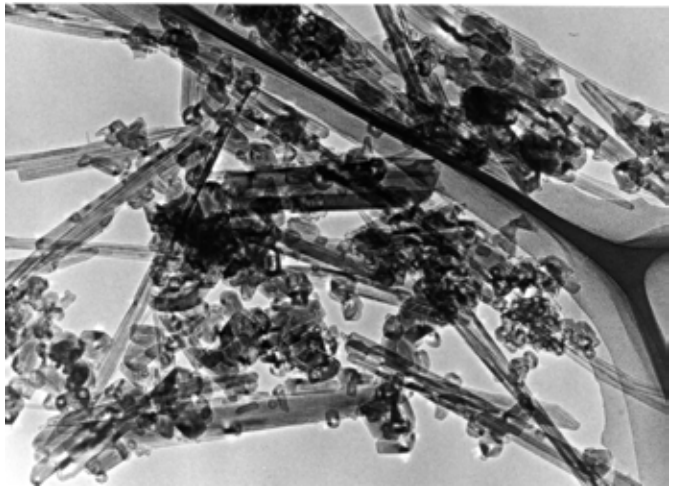


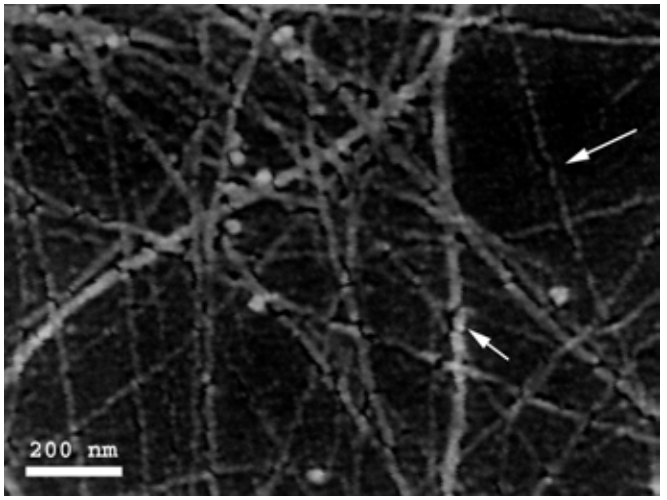
Multi-Walled Carbon Nanotubes (MWCNTs)





What CNTs Really Look Like!





- Clumps, ropes, bundles, mats
- Residual catalyst, other carbon forms
- Very high tendency to stick together

Are CNTs Just Graphite Toxicologically?

MATERIAL SAFETY DATA SHEET

Manufacturer: [redacted]

Product: [redacted] **Carbon Nanotubes**

Section 1 Product Identification

Chemical Name:	Carbon Fullerene
Formula:	Carbon
Chemical Family:	Synthetic Graphite
Synonyms:	Carbon Nanotubes
CAS Number:	7782-42-5 (Graphite)

Section 2 Composition and Information on Ingredients

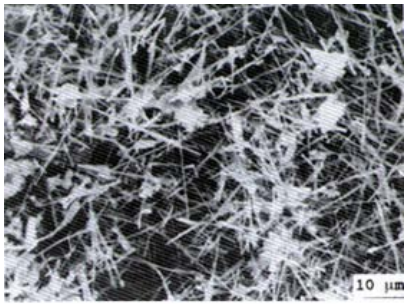
Component	%	OSHA/PEL	ACGIH/TLV
Synthetic graphite	Up to 100%	15 mg/m^3 (total dust) 5 mg/m^3 (respirable fraction)	2 mg/m^3 TWA
Metallic impurity	Balance		

- **Actual MSDS for CNTs from *****
- **Quoted PEL is for graphite!**

Fiber Toxicology

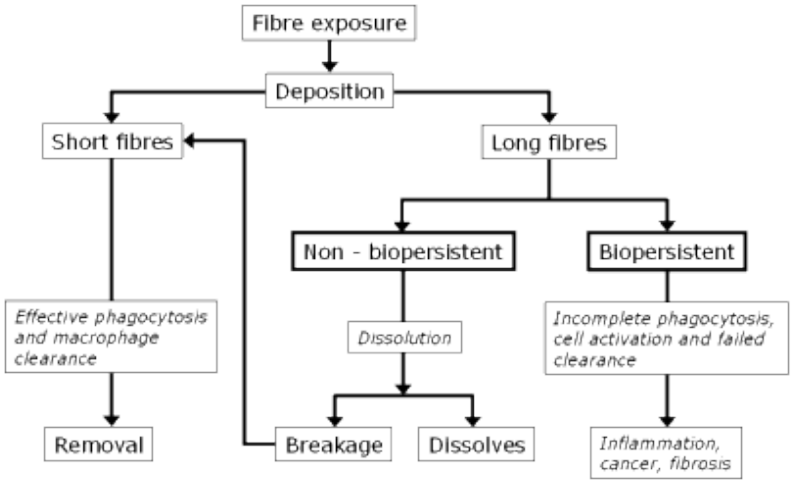
- **Many naturally occurring and man-made fibers can induce mesothelioma, lung cancer and/or pulmonary fibrosis:**
  - **Fibrous erionite & zeolite: High rate of mesothelioma in the Anatoly region of Turkey where they occur naturally–more potent than asbestos**
  - **Man made vitreous fibers**
    Man made refractory ceramic fibers
  - **Silicon carbide whiskers: Similar potency to asbestos**
  - **Aluminum oxide, attapulgite, dawsonite, potassium titanate**





Fiber Toxicology

- **Key factors contributing to toxicity:**
  - **Diameter < 1000 nm**
  - **Length >5,000 nm:**
  - **High biopersistance**
  - **Poor pulmonary clearance**
- **Chemistry probably matters too a lesser degree**



The three D's: Dose, dimensions and durability!

What has been published about carbon nanotube toxicity?

Pulmonary Toxicity: All Published *Instillation/Aspiration Studies* up to Mid 2008

Year	Author	Species	Granuloma	Inflamm	Diffuse Fibrosis	Death	Other
2001	Huczko	Guinea pig	–	No	–	No	
2004	Warheit	Rat	Yes[a]	Yes[b]	No	Yes[c]	
2004	Lam	Rat	Yes	Yes	–	Yes[c]	
2005	Muller	Rat	Yes	Yes	Yes	Yes	Biopersistent
2005	Huczko (Grubek-Jaworska)	Guinea pig	Yes	Yes	Yes	No	Pneumonitis
2005	Shvedova	Mouse	Yes	Yes[b]	Yes[d]	No	Odx. Stress
2006	Mangum	Rats	Yes	No	Yes	No	
2006	Carrero Sanchez	Mice	Yes	Yes	–	Yes	
2007	Shevdova	Mice	Yes	Yes	Yes	Yes	Oxd. Stress, esp. w/o vit. E
2008	Han	Mice	–	Yes	–	–	

A = non-uniform, b = transient, c = by choking, d = progressive

Conclusions from "Better" Instillation/Aspiration Studies

- **Lam (NASA) 2004**
  - **"…*if nanotubes reach the lungs, they are much more toxic than carbon black and can be more toxic than quartz*…"**
- **Shvedova (NIOSH), 2005**
  - **"…if workers are exposed to respirable SWCNT particles at the current PEL (for graphite particles) they may be at risk of developing some lung lesions."**
- **Muller (Belgium), 2005**
  - **"…if multiwalled carbon nanotubes reach the lung they are biopersistent…and induce lung inflammation and fibrosis."**
- **Huczko (Poland), 2005**
  - **"…CNTs should be considered a serious occupational health hazard…"**
- **Magnum (CIIT) 2006**

"…SWCNTs … induce the formation of small, focal interstitial fibrotic lesions in the alveolar regions of the lungs of rats"

First NIOSH Inhalation Study

Am J Physiol Lung Cell Mol Physiol (July 25, 2008). doi:10.1152/ajplung.90287.2008
Submitted on April 23, 2008
Revised on June 24, 2008
Accepted on July 18, 2008

INHALATION VERSUS ASPIRATION OF SINGLE WALLED CARBON NANOTUBES IN C57BL/6 MICE: INFLAMMATION, FIBROSIS, OXIDATIVE STRESS AND MUTAGENESIS

Anna A. Shvedova[1*], Elena R. Kisin[1], Ashley R. Murray, Victor J Johnson[2], Olga Gorelik[3], Sivaram Arepalli[3], Ann F. Hubbs[1], Robert R. Mercer[1], Phouthone Keohavong[4], Nancy Sussman[4], J. Jin[4], J. Yin[4], Samuel Stone[1], Bean T Chen[1], Gregory Deye[1], Andrew Maynard[5], Vincent Castranova[1], Paul A. Baron[1], and Valerian E Kagan[4]

[1] NIOSH
[2] National Institute for Occupational Safety and Health
[3] NASA
[4] University of Pittsburgh
[5] Woodrow Wilson Center

- **2-4x more lung inflammation than aspiration**
- **2-4x more diffuse fibrosis and granuloma formation**

- ***"Based on the outcomes of our inhalation study, it could be inferred that if workers were subjected to long-term exposures to respirable SWCNT at the current PEL for synthetic graphite, they would likely have increased risk for pulmonary changes***

Same Story from BASF

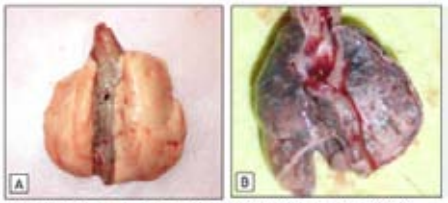
INHALATION TOXICITY OF MULTI-WALL CARBON NANOTUBES IN RATS EXPOSED FOR 3 MONTHS.

Ma-Hock L, Treumann S, Strauss V, Brill S, Luizi F, Mertler M, Wiench K, Gamer AO, van Ravenzwaay B, Landsiedel R.

Product Safety, BASF SE, 67056 Ludwigshafen, Germany.

Carbon nanotubes (CNT) are of great commercial interest. Theoretically, during processing and handling of CNT and in abrasion processes on composites containing CNT, inhalable CNT particles might be set free. For hazard assessment, we performed a 90-day inhalation toxicity study with a multi-wall CNT (MWCNT) material (Nanocyl NC 7000) according to OECD test guideline 413. Wistar rats were head-nose exposed for 6 hours/day, 5 days/week, 13 weeks, total 65 exposures, to MWCNT concentrations of 0 (control), 0.1, 0.5 or 2.5 mg/m(3). Highly respirable dust aerosols were produced with a proprietary brush generator which neither damaged the tube structure nor increased reactive oxygen species on the surface. Inhalation exposure to MWCNT produced no systemic toxicity. However, increased lung weights, pronounced multifocal granulomatous inflammation, diffuse histiocytic and neutrophilic inflammation, and intra-alveolar lipoproteinosis were observed in lung and lung-associated lymph nodes at 0.5 and 2.5 mg/m(3). These effects were accompanied by slight blood neutrophilia at 2.5 mg/m(3). Incidence and severity of the effects were concentration-related. At 0.1 mg/m(3), there was still minimal granulomatous inflammation in the lung and in lung-associated lymph nodes; a no observed effect concentration was therefore not established in this study. The test substance has low dust-forming potential, as demonstrated by dustiness measurements, but nonetheless strict industrial hygiene measures must be taken during handling and processing. Toxicity and dustiness data such as these can be used to compare different MWCNT materials and to select the material with the lowest risk potential for a given application.

PMID: 19584127 [PubMed - as supplied by publisher]

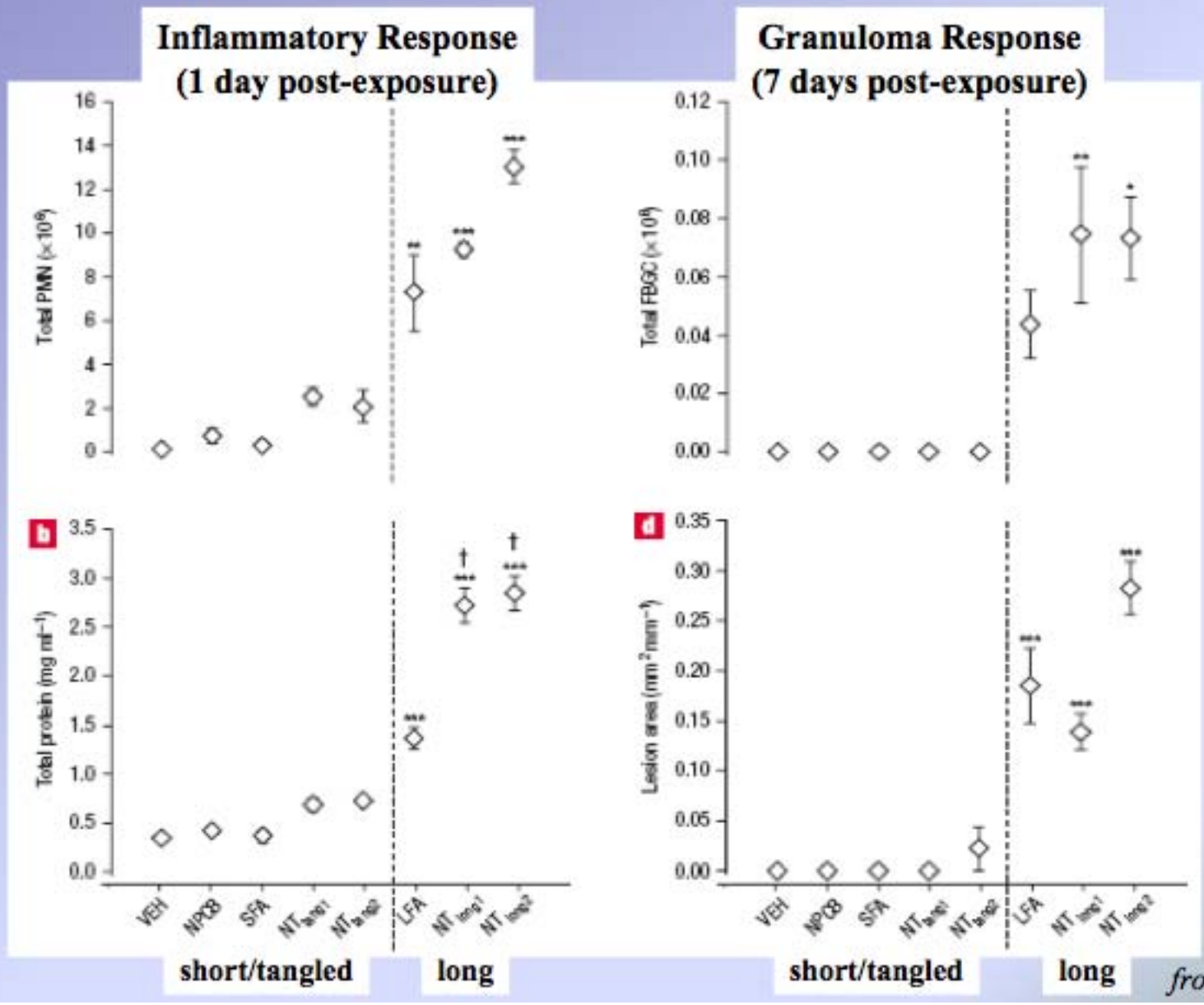


Publication of the study has sparked criticism from US NGO Environmental Defense Fund which notes that when BASF notified the product to the US Environmental Protection Agency last year, it claimed confidentiality over the identity of the product, even whether it was single or multi-walled, yet it is now prepared to discuss such details in a scientific journal.

Do MW-CNTs Behave Like Asbestos in *In Vivo* Studies?

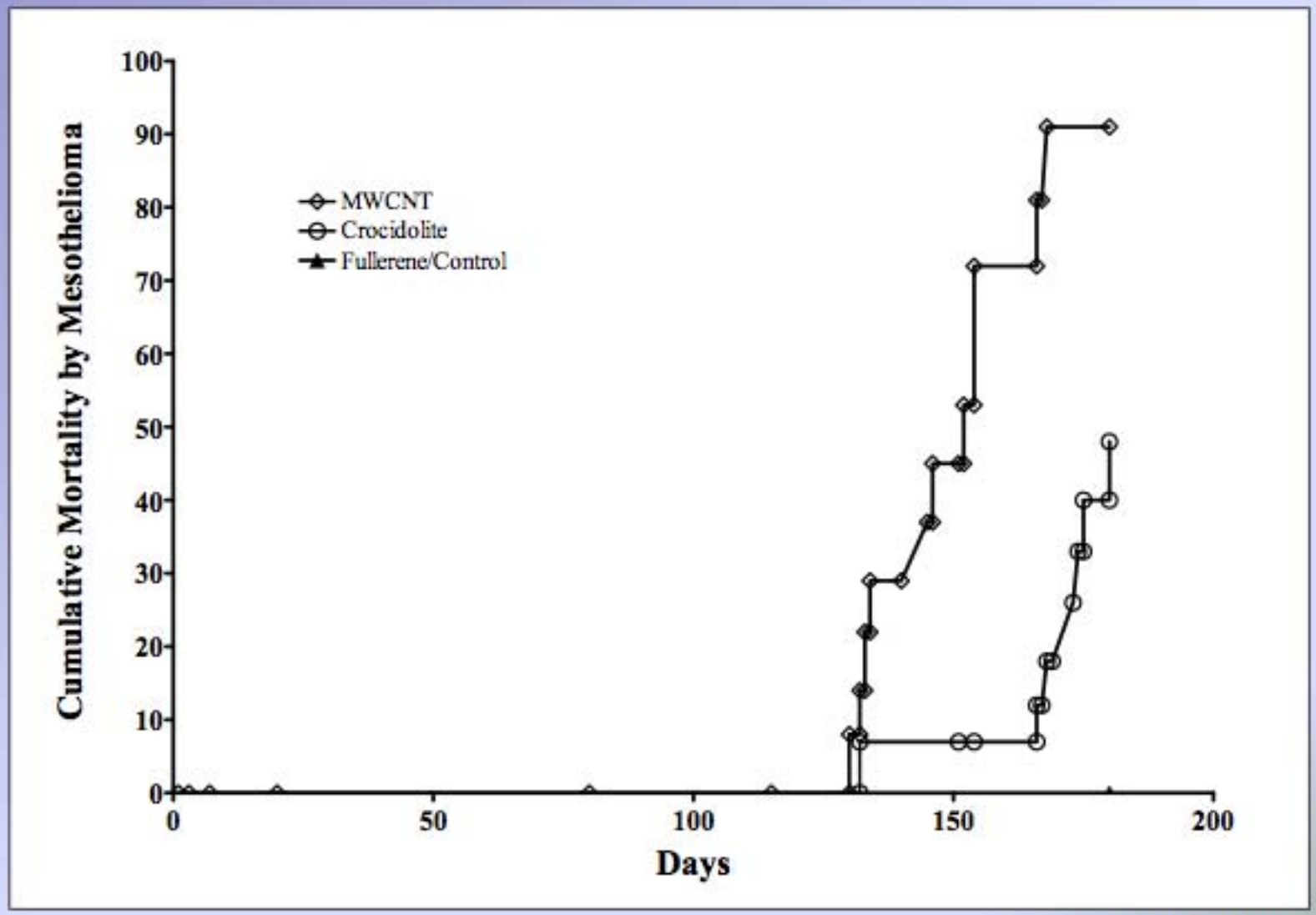
1. Poland et al., 2008 (*Nature Nanotechnol.*)
   - Intraperitoneal injections (50 μg) in mice;
   - Compared 4 CNT preparations to carbon black and short and long amosite fibers.
2. Takagi et al., 2008 (*J. Toxicol. Sci.*)
   - Intraperitoneal injections (3 mg) in $p53^{+/-}$ mice;
   - Compared to crocidolite fibers and fullerene.

Do MW-CNTs Behave Like Asbestos?



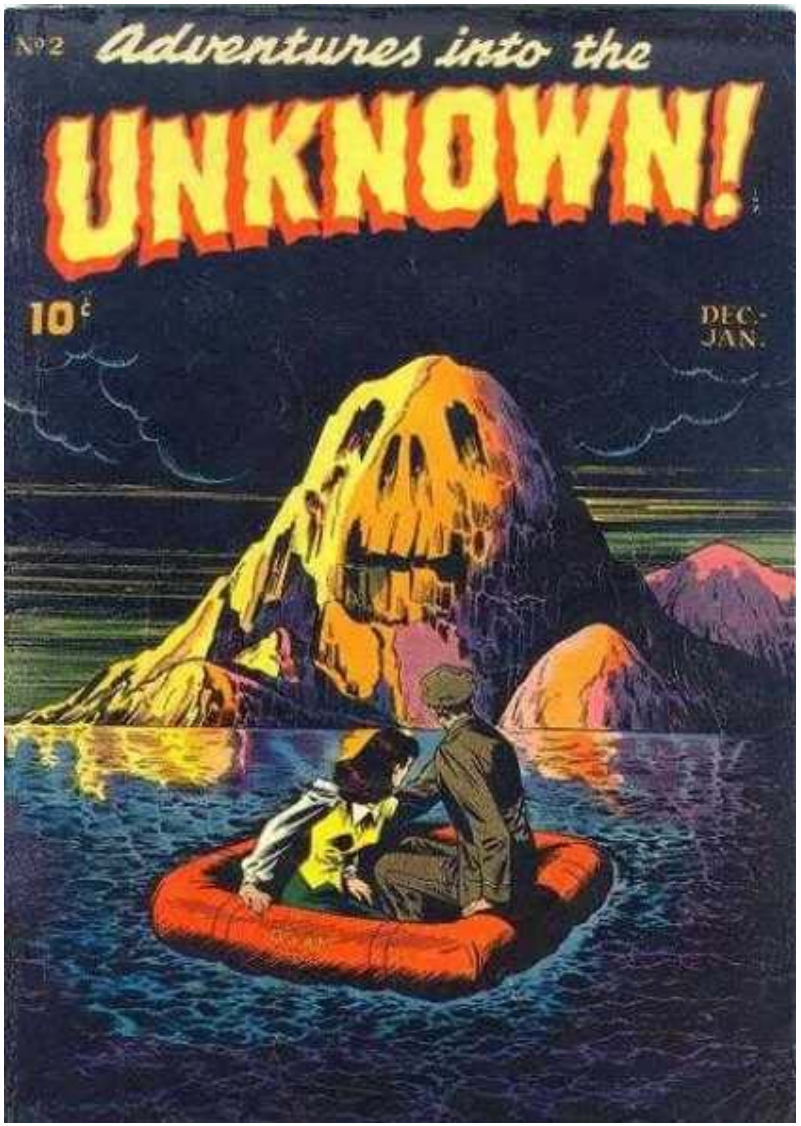
from Poland et al., 2008

Do MW-CNTs Behave Like Asbestos in p53$^{+/-}$ Mice?



Adapted from Takagi et al., 2008

Many Unknown



- Chronic toxicity??
- Reproductive toxicity??
- Neurotoxicity??
- Hepatotoxicity??
- Endocrine disruption??

Questions?